2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of ethylbenzene. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health. A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure- inhalation, oral, and dermal; and then by health effect-death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods-acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observedadverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR belieyes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear.

LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of ethylbenzene are indicated in Table 2- 1 and Figure 2- 1.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for ethylbenzene. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

2.2.1 Inhalation Exposure

2.2.1.1 Death

No studies were located regarding lethality in humans following only inhalation exposure to ethylbenzene. Matsumoto et al. (1992) reported the case of a 44-year-old man was found unconscious in his gasoline vapor-filled car with his clothes wet with gasoline containing ethylbenzene among many other constituents. The patient emptied at least 18 L of gasoline into his car and was exposed to it for 10 hours more. He was diagnosed as suffering from chemical burns. He denied ingestion of gasoline, and no evidence of gasoline ingestion was found on examination. The patient died after 9 days of multiple organ failure. Ethylbenzene was detectable in his blood. However, it was not possible to determine the extent to which his death was due to exposure to ethylbenzene versus the other components of the gasoline.

The LC_{50} (lethal concentration, 50% kill) for rats following inhalation exposure to ethylbenzene is reported to be 4,000 ppm (Smyth et al. 1962) and 13,367 ppm (Ivanov 1962) following exposure durations of 4 and 2 hours, respectively. The doses required to cause 100% mortality in rats were shown to be 8,000 ppm (Smyth et al. 1962) and 16,698 ppm (Ivanov 1962) for 4- and 2-hour inhalation exposures, respectively. However, it is important to note that the results of both of these studies have limited utility because the recorded concentrations were not analytically verified. Although no studies were located regarding the effect of nutritional status on mortality, it has been postulated that food deprivation may decrease ethylbenzene toxicity since the detoxication of ethylbenzene is increased significantly in fasted rats (Nakajima and Sato 1979).

The lethality of ethylbenzene in animals following inhalation exposure has been shown to vary among species. This was demonstrated in a study using Fischer 344 rats, $B6C3F_1$ mice, and New Zealand White rabbits in which the animals were exposed to 0,400, 1,200, or 2,400 ppm ethylbenzene 6 hours a day for 4 days (Biodynamics 1986; Cragg et al. 1989). Mortality occurred in mice at half the dose (1,200 ppm) required to cause death in rats (2,400 ppm). All rabbits in each exposure group survived.

Wolf et al. (1956) evaluated the toxicity of ethylbenzene in Rhesus monkeys after inhalation exposure 7-8 hours a day, 5 days a week for 6 months. Two female Rhesus monkeys were exposed to 400 ppm ethylbenzene, and a male and female monkey were exposed to 600 ppm. No mortality was reported. Similarly, no mortality was reported by Wolf et al. (1956) in male and female rats (strain unspecified)

exposed to 0, 400, 600, or 1,250 ppm ethylbenzene, or male rats exposed to 2,200 ppm ethylbenzene in a similar study design. No mortality was observed in a 4-week inhalation study in which Fischer 344 rats and B6C3F$_1$ mice were exposed to 99,382, or 782 ppm ethylbenzene, 6 hours a day, 5 days a week; all New Zealand White rabbits survived after exposure to 1,610 ppm ethylbenzene in the same study (Cragg et al. 1989). In a 90-day study, no lethality was observed in Fischer 344/N rats and B6C3F1 mice exposed to 0,99,246,498,740, or 975 ppm ethylbenzene, 6 hours a day, 5 days a week (NTP 1992). Wolf et al. (1956) observed no mortality in male or female guinea pigs exposed to 0,400,600, or 1,250 (females only) ppm ethylbenzene; or rabbits exposed to 0,400, or 600 ppm (for males), or 0,400, 600, or 1,250 ppm (for females) ethylbenzene for 5 days a week, 7-8 hours a day for up to 6-7 months. Chronic-duration inhalation exposure of male and female Fischer 344 rats and B6C3F$_1$ mice to doses of 0,75,250, or 750 ppm for up to 2 years (103-104 weeks) revealed increased mortality (96% mortality) in male rats exposed to 750 ppm (NTP 1996). Male and female rats and mice in the other dose groups had mortality rates that did not differ significantly from the control group.

The LC$_{50}$ values and all reliable LOAEL values for death in rats and mice following acute- or chronicduration exposure are recorded in Table 2- 1 and plotted in Figure 2- 1.

2.2.1.2 Systemic Effects

Data are limited on the systemic effects of inhaled ethylbenzene in humans. Most of the information available is from case reports in which quantitative data on exposure concentrations and durations were not reported. In addition, most of these studies lack important study details or have confounding factors (e.g., simultaneous exposures to other toxic substances). In general, the systemic effects observed in humans were pulmonary and ocular irritation, and possible hematological alterations (Angerer and Wulf 1985; Cometto-Muniz and Cain 1995; Thienes and Haley 1972; Yant et al. 1930).

Several studies were located on the systemic effects of ethylbenzene in animals following inhalation exposure. The principal target organs appear to be the lungs, liver, and kidney, with transient toxic effects on the hematological system. However, no definitive conclusions can be drawn because of the limitations of many of the studies.

Table 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	

ACUTE EXPOSURE

Death

1	Rat (Fischer- 344)	4 d 6 hr/d				2400 M (100% mortality by day 3)	Biodynamics 1986; Cragg et al. 1989
2	Rat (NS)	4 hr				4000 M (LC$_{50}$)	Smyth et al. 1962
3	Mouse (B6C3F1)	4 d 6 hr/d				1200 M (4/5 animals died by day 3)	Biodynamics 1986; Cragg et al. 1989

Systemic

4	Rat (Fischer- 344)	4 d 6 hr/d	Resp	1200 M		2400 M (shallow breathing)	Biodynamics 1986; Cragg et al. 1989
			Hepatic		400M (increased liver weight)		
			Renal	400 M	1200M (increased relative kidney weight)		
			Ocular	400 M	1200M (lacrimation)		
			Bd Wt	1200 M			
			Other	400 M	1200M (yellow or brown anogenital staining)		

Table 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
5	Rat (Sprague-Dawley)	3 d 6 hr/d	Hepatic		2000M (increased liver-to-body weight ratio, increased cytochrome P-450 conc & increased NADPH-cytochrome C reductase activity; increased 7-ethyoxyresorufin, n-hexane hydroxylation & benz[a]pyrene)		Toftgard and Nilsen 1982
			Renal		2000M (increased kidney-to-body weight ratio & increased NADPH-cytochrome C reductase activity)		
6	Mouse (B6C3F1)	4 d 6 hr/d	Resp	400 M		1200 M (shallow breathing)	Biodynamics 1986; Cragg et al. 1989
			Hepatic	1200 M			
			Renal	1200 M			
			Ocular		400M (lacrimation)		
			Bd Wt	400 M			
7	Mouse (Swiss)	5 min	Resp		1432M (RD50; 50% respiratory depression due to sensory irritation)		De Ceaurriz et al. 1981
8	Mouse (Swiss-Webster)	30 min	Resp		4060M (50% respiratory depression)		Nielsen and Alarie 1982
9	Mouse (CFW)	20 min	Ocular		2000M (lacrimation & palpebral closure)		Tegeris and Balster 1994

Table 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
10	Rabbit (New Zealand)	4 d 6 hr/d	Resp	2400 M			Biodynamics 1986; Cragg et al. 1989
			Hepatic	2400 M			
			Renal	2400 M			
			Ocular		400 M (lacrimation)		
			Bd Wt	2400 M			

Neurological

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	Less serious (ppm)	Serious (ppm)	Reference
11	Rat (Sprague-Dawley)	3 d 6 hr/d			2000 M (neurotransmission disturbance in the forebrain & hypothalmus)		Andersson et al. 1981
12	Rat (Fischer- 344)	4 d 6 hr/d		1200 M		2400 M (salivation, prostration)	Biodynamics 1986; Cragg et al. 1989
13	Rat (CFY)	4 hr		200 M	400 M (moderate activation in motor behavior)	2180 M (narcotic effects)	Molnar et al. 1986
14	Mouse (B6C3F1)	4 d 6 hr/d		400 M		1200 M (prostration & reduced activity)	Biodynamics 1986; Cragg et al. 1989
15	Mouse (CFW)	20 min			2000 M (posture changes, decreased arousal & rearing, disturbed gait, decreased mobility, righting reflex, decreased grip strength, increased landing foot splay, impaired psychomotor coordination)		Tegeris and Balster 1994

Table 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
16	Rabbit (New Zealand)	4 d 6 hr/d		2400 M			Biodynamics 1986; Cragg et al. 1989
17	Rabbit (New Zealand)	7 d 12 hr/d			750 M (dopamine depletion)		Mutti et al. 1988
18	Rabbit (New Zealand)	7 d 12 hr/d			750 M (dopamine depletion; HVA accumulation)		Romanelli et al. 1986
Reproductive							
19	Rat (Fischer- 344)	4 d 6 hr/d		2400 M			Biodynamics 1986; Cragg et al. 1989
20	Rat (CFY)	Gd 7-15 24 hr/d				138 (resorptions)	Ungvary and Tatrai 1985
21	Mouse (B6C3F1)	4 d 6 hr/d		1200 M			Biodynamics 1986; Cragg et al. 1989
22	Mouse (CFLP)	Gd 6-15 24 hr/d		115 F			Ungvary and Tatrai 1985
23	Rabbit (New Zealand)	4 d 6 hr/d		2400 M			Biodynamics 1986; Cragg et al. 1989
24	Rabbit (New Zealand)	Gd 6-16 24 hr/d				230 F (abortions)	Ungvary and Tatrai 1985

Table 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
Developmental							
25	Rat (CFY)	Gd 7-15 6 hr/d		138 F			Ungvary and Tatrai 1985
26	Rat (CFY)	Gd 7-15 24 hr/d				138 F (skeletal retardation & fetal resorptions)	Ungvary and Tatrai 1985
27	Mouse (CFLP)	Gd 6-15 24 hr/d				115 F (anomalies of uropoetic apparatus)	Ungvary and Tatrai 1985
28	Rabbit (New Zealand)	Gd 7-20 24 hr/d			115 F (decreased fetal weight)		Ungvary and Tatrai 1985
INTERMEDIATE EXPOSURE							
Systemic							
29	Rat (Wistar)	3 wk 5 d/wk 7 hr/d Gd 1-19 7 hr/d	Resp	985 F			Andrew et al. 1981
			Hepatic	97	959 F (increased liver weight)		
			Renal	97	959 F (increased kidney weight)		
			Bd Wt	985 F			
			Other	985 F			

Table 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL				Reference
					Less serious (ppm)		Serious (ppm)		
30	Rat (Fischer- 344)	4 wk 5 d/wk 6 hr/d	Resp	782					Cragg et al. 1989
			Cardio	782					
			Gastro	782					
			Hemato	382	782	(increased platelet counts in male; increased mean total leukocyte counts in male & females)			
			Musc/skel	782					
			Hepatic	382	782	(increased absolute & relative liver weight)			
			Renal	782					
			Endocr	782					
			Ocular	99	382	(sporadic incidence of lacrimation)			
			Bd Wt	782					
31	Rat (Wistar)	5-16 wk 5 d/wk 6 hr/d	Hepatic	300 M	600M	(increased relative liver weight 10% at wk 9; increased microsomal protein content, NADPH -cytochrome C reductase activity, 7-ethoxy-coumarin O-diethylase & UDPG-transferase)			Elovaara et al. 1985
			Renal	300 M	600M	(relative kidney weight increase 10% at wk 9)			
			Bd Wt	600					

Table 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
32	Rat (F344/N)	13 wk 5 d/wk 6 hr/d	Resp	740 M	975 M (increased relative lung weight)		NTP 1992
				99.4 F	246 F (increased absolute & relative lung weight)		
			Cardio	975			
			Gastro	975			
			Hemato	975			
			Musc/skel	975			
			Hepatic	99.4 M	246 M (increased absolute and relative liver weight)		
				246 F	498 F (increased absolute liver weight)		
			Renal	246 M	498 M (increased absolute & relative kidney weight		
				498 F	740 F <10% (increased absolute kidney weight <10%)		
			Endocr	975			
			Ocular	975			
			Bd Wt	975			

Table 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
33	Mouse (B6C3F1)	4 wk 5 d/wk 6 hr/d	Resp	782			Cragg et al. 1989
			Cardio	782			
			Gastro	782			
			Hemato	782			
			Musc/skel	782			
			Hepatic	382	782 (increased mean absolute and relative liver weight)		
			Renal	782			
			Endocr	782			
			Ocular	782			
			Bd Wt	782			
34	Mouse (B6C3F1)	13 wk 5 d/wk 6 hr/d	Resp	975			NTP 1992
			Cardio	975			
			Gastro	975			
			Hemato	975			
			Musc/skel	975			
			Hepatic	498 M	740 M (increased absolute and relative liver weight)		
				740 F	975 F (increased absolute & relative liver weight)		
			Renal	975 M 740 F	975 F (increased relative kidney weight)		
			Endocr	975			
			Ocular	975			
			Bd Wt	975			

Table 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
35	Rabbit (New Zealand)	Gd 1-24 7 d/wk 7 hr/d	Resp	962 F			Andrew et al. 1981
			Hepatic	99 F	962 F (increased absolute & relative liver weights in pregnant rabbits)		
			Renal	962 F			
			Bd Wt	962 F			
			Other	962 F			
36	Rabbit (New Zealand)	4 wk 5 d/wk 6 hr/d	Resp	1610			Cragg et al. 1989
			Cardio	1610			
			Gastro	1610			
			Hemato	1610			
			Musc/skel	1610			
			Hepatic	1610			
			Renal	1610			
			Endocr	1610			
			Ocular	1610			
			Bd Wt	1610			

Immunological/Lymphoreticular

Key to[a] figure	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	Less serious (ppm)	Serious (ppm)	Reference
37	Rat (Wistar)	3 wk 5 d/wk 7 hr/d Gd 1-19 7 hr/d		97 F	959 F (increased spleen weight)		Andrew et al. 1981
38	Rat (Fischer- 344)	4 wk 5 d/wk 6 hr/d		782			Cragg et al. 1989

Table 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
39	Rat (F344/N)	13 wk 5 d/wk 6 hr/d		975			NTP 1992
40	Mouse (B6C3F1)	4 wk 5 d/wk 6 hr/d		782			Cragg et al. 1989
41	Mouse (B6C3F1)	13 wk 5 d/wk 6 hr/d		975			NTP 1992
42	Rabbit (New Zealand)	4 wk 5 d/wk 6 hr/d		1610			Cragg et al. 1989
Neurological							
43	Rat (Fischer- 344)	4 wk 5 d/wk 6 hr/d		99	382 (sporadic incidence of salivation)		Cragg et al. 1989
44	Rat (F344/N)	13 wk 5 d/wk 6 hr/d		975			NTP 1992
45	Mouse (B6C3F1)	4 wk 5 d/wk 6 hr/d		782			Cragg et al. 1989
46	Mouse (B6C3F1)	13 wk 5 d/wk 6 hr/d		975			NTP 1992
47	Rabbit (New Zealand)	4 wk 5 d/wk 6 hr/d		1610			Cragg et al. 1989

Table 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
Reproductive							
48	Rat (Wistar)	3 wk 5 d/wk 7 hr/d Gd 1-19 7 hr/d		985 F			Andrew et al. 1981
49	Rat (Fischer- 344)	4 wk 5 d/wk 6 hr/d		782			Cragg et al. 1989
50	Rat (F344/N)	13 wk 5 d/wk 6 hr/d		975			NTP 1992
51	Mouse (B6C3F1)	4 wk 5 d/wk 6 hr/d		782			Cragg et al. 1989
52	Mouse (B6C3F1)	13 wk 5 d/wk 6 hr/d		975			NTP 1992
53	Rabbit (New Zealand)	Gd 1-24 7 d/wk 7 hr/d		962 F			Andrew et al. 1981
54	Rabbit (New Zealand)	4 wk 5 d/wk 6 hr/d		1610			Cragg et al. 1989

Table 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)		LOAEL Serious (ppm)	Reference
Developmental								
55	Rat (Wistar)	3 wk 5 d/wk 7 hr/d Gd 1-19 7 hr/d		97[b]	959	(skeletal anomalies, supernumerary ribs)		Andrew et al. 1981
56	Rabbit (New Zealand)	Gd 1-24 7 d/wk 7 hr/d		962				Andrew et al. 1981
CHRONIC EXPOSURE								
Death								
57	Rat (F344/N)	104 wk 5 d/wk 6 hr/d					750 M (2/50 survived)	NTP 1996
Systemic								
58	Rat (F344/N)	104 wk 5 d/wk 6 hr/d	Resp	750				NTP 1996
			Cardio	750				
			Gastro	750				
			Musc/skel	750				
			Hepatic	750				
			Renal	250	750	(renal tubule hyperplasia)		
			Endocr	750				
			Bd Wt	750				

Table 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
59	Mouse (B6C3F1)	103 wk 5 d/wk 6 hr/d	Resp	250 F	750M (alveolar epithelial metaplasia)		NTP 1996
			Cardio	750			
			Gastro	750			
			Musc/skel	750			
			Hepatic	250	750F (syncytial alteration, hypertrophy, necrosis, eosinophilic focus)		
			Renal	750			
			Endocr	250	750 (follicular cell hyperplasia in thyroid gland)		
			Bd Wt	750			
Immunological/Lymphoreticular							
60	Rat (F344/N)	104 wk 5 d/wk 6 hr/d		750			NTP 1996
61	Mouse (B6C3F1)	103 wk 5 d/wk 6 hr/d		750			NTP 1996
Neurological							
62	Rat (F344/N)	104 wk 5 d/wk 6 hr/d		750			NTP 1996
63	Mouse (B6C3F1)	103 wk 5 d/wk 6 hr/d		750			NTP 1996

Table 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
Reproductive							
64	Rat (F344/N)	104 wk 5 d/wk 6 hr/d		250 M 750 F		750 M (interstitial cell adenoma, bilateral testicular adenoma)	NTP 1996
65	Mouse (B6C3F1)	103 wk 5 d/wk 6 hr/d		750			NTP 1996
Cancer							
66	Rat (F344/N)	104 wk 5 d/wk 6 hr/d				750 (CEL: renal tubule adenoma or carcinoma, M: 21/50, F: 8/49; males: 44/50 testicular adenoma)	NTP 1996
67	Mouse (B6C3F1)	103 wk 5 d/wk 6 hr/d				750 M CEL: alveolar/bronchiolar adenoma, 16/50, alveolar/bronchiolar adenoma or carcinoma, 19/50) 750 F (CEL: hepatocellular adenoma, 16/50; hepatocellular adenoma or carcinoma, 25/50)	NTP 1996

[a]The number corresponds to entries in Figure 2-1.

[b]Used to derive an intermediate inhalation MRL of 1.0 ppm. Concentration divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; F = female; Gastro = gastrointestinal; Gd = gestational day; Hemato = hematological; hr = hour(s); HVA = homovanillic acid; LC_{50} = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; Metab = metabolic; min = minute(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; RD_{50} = respiratory depression, 50%; Resp = respiratory; wk = week(s)

Figure 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation

Acute (≤14 days)

Key

r	rat
m	mouse
h	rabbit

■ LC$_{50}$ (animals)

● LOAEL for serious effects (animals)

◐ LOAEL for less serious effects (animals)

○ NOAEL (animals)

◆ CEL: cancer effect level (animals)

Minimal risk level
for effects other
than cancer

The number next to each
point corresponds to
entries in Table 2-1.

* Doses represent the lowest dose tested per study that produced a tumorigenic response
and do not imply the existence of a threshold for the cancer end point.

Figure 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (cont.)

Intermediate (15-364 days)

Systemic

Respiratory Cardiovascular Gastrointestinal Hematological Musculoskeletal

(ppm)

10000

1000
  29r 30r   33m 34m 35h 36h

100
  32r

10

1

0.1

Key		
r rat m mouse h rabbit	■ LC_{50} (animals) ● LOAEL for serious effects (animals) ◐ LOAEL for less serious effects (animals) ○ NOAEL (animals) ◆ CEL: cancer effect level (animals)	¦ Minimal risk level ¦ for effects other ѡ than cancer The number next to each point corresponds to entries in Table 2-1.

* Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer end point.

Figure 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (cont.)

Intermediate (15-364 days)

Systemic

					Key			

r	rat	■ LC$_{50}$ (animals)	¦ Minimal risk level
m	mouse	● LOAEL for serious effects (animals)	¦ for effects other
h	rabbit	◐ LOAEL for less serious effects (animals)	⋃ than cancer
		○ NOAEL (animals)	The number next to each *point corresponds to*
		◆ CEL: cancer effect level (animals)	entries in Table 2-1.

* Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer end point.

Figure 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (cont.)

Intermediate (15-364 days)
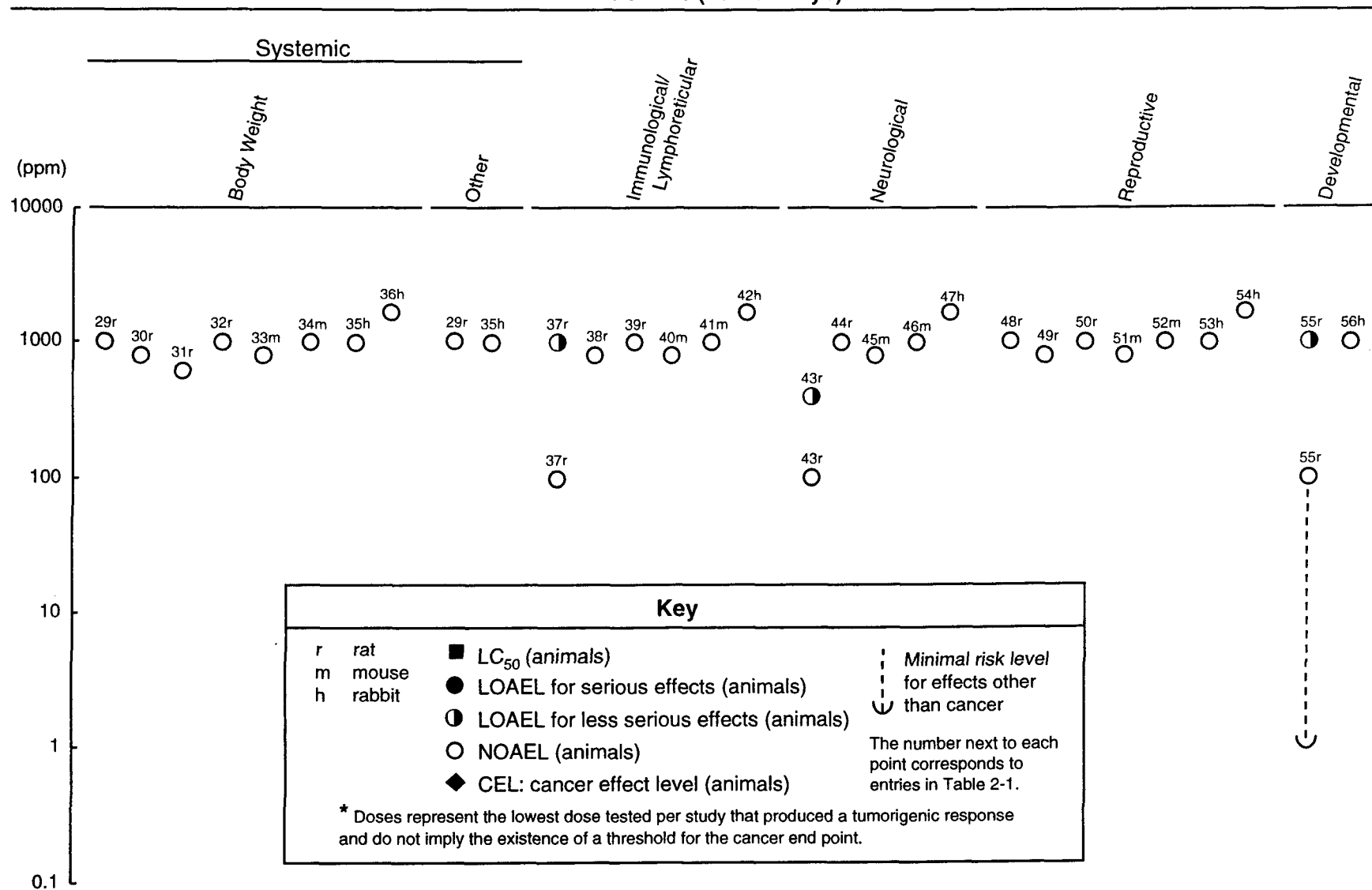
Figure 2-1. Levels of Significant Exposure to Ethylbenzene - Inhalation (cont.)

Chronic (≥365 days)

Key

r	rat
m	mouse
h	rabbit

■ LC₅₀ (animals)

● LOAEL for serious effects (animals)

◖ LOAEL for less serious effects (animals)

○ NOAEL (animals)

◆ CEL: cancer effect level (animals)

Minimal risk level for effects other than cancer

The number next to each point corresponds to entries in Table 2-1.

* Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer end point.

No studies were located describing cardiovascular, gastrointestinal, musculoskeletal, renal, endocrine, dermal, body weight, or metabolic effects in humans, or dermal effects in animals after inhalation exposure to ethylbenzene.

The systemic effects observed after inhalation exposure are discussed below. The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 2- 1 and plotted in Figure 2- 1.

Respiratory Effects. Throat irritation and chest constriction were reported in the six male volunteers acutely exposed to levels of ethylbenzene in the air as low as 2,000 ppm (Yant et al. 1930). Symptoms became more extreme following exposure to 5,000 ppm. No other significant respiratory changes were reported. The utility of these results is limited because the exposure durations necessary for these effects to occur were not clearly described and the ethylbenzene used for testing reportedly contained small amounts of impurities (e.g., benzol and diethylbenzene). In addition, the methods used to calculate the actual vapor concentration of ethylbenzene were not well described, making it difficult to determine the accuracy of the methods. In case studies involving a male and a female patient, no respiratory effects were observed when the patients were exposed to 55.3 ppm ethylbenzene for 15 minutes in an inhalation chamber (Moscato et al. 1987).

Microscopic examination of tissues from Fischer 344 rats and $B6C3F_1$ mice exposed to 1,200-2,400 ppm ethylbenzene via inhalation for 4 days showed pulmonary congestion in animals that had died. However, no information on the cause of death was provided; therefore, it is not known if these effects are treatment-related (Biodynamics 1986; Cragg et al. 1989). No effects were seen in Sprague-Dawley rats exposed to 2,000 ppm for 3 days (Toftgard and Nilsen 1982) or New Zealand White rabbits exposed to doses up to 2,400 ppm for 4 days (Biodynamics 1986; Cragg et al. 1989). The concentration of ethylbenzene required to decrease the respiratory rate in mice by 50% (RD_{50}) after inhalation exposure has been determined (De Ceaurriz et al. 1981; Nielsen and Alarie 1982). These values were reported to be 1,432 ppm in male Swiss OFi mice (De Ceaurriz et al. 1981) and 4,060 ppm in Swiss Webster mice exposed to ethylbenzene (Nielsen and Alarie 1982). Respiratory depression was also observed in Swiss Webster mice by Nielsen and Alarie (1982) after intratracheal administration of 4,000 ppm ethylbenzene for 30 minutes. Guinea pigs (strain unspecified) were exposed to ethylbenzene vapor at various concentrations and acute durations (Yant et al. 1930). Nasal irritation was observed in animals exposed to 1,000 ppm for 8 and 3 minutes and in animals exposed to 2,000, 5,000, and 10,000 ppm for 480, 30, and 10 minutes, respectively. Gross

histopathology revealed congestion and edema in the lungs, with an increase in the severity of damage with increased exposure concentration (dose not specified).

Andrew et al. (198 1) investigated the teratologic effects of ethylbenzene exposures in Wistar rats exposed to 0,97, or 959 ppm ethylbenzene for 7 hours a day, 5 days a week for 3 weeks. They were then mated and exposed to ethylbenzene (0,96, or 985 ppm) 7 hours a day, 7 days a week through gestational day (Gd) 19. The rats were then sacrificed and examined at Gd 21. No adverse effects on lung histopathology were found. New Zealand White rabbits dosed at the same levels for 3 weeks prior to mating and after mating until Gd 24 also exhibited no adverse histopathological effects on lung tissue at evaluation on Gd 30. No adverse pulmonary effects were reported when B6C3F$_1$ mice (Cragg et al. 1989) and Fischer 344 rats (Cragg et al. 1989) were exposed to concentrations of ethylbenzene at 782 ppm for 4 weeks. New Zealand White rabbits exposed to concentrations as high as 1,610 ppm for 4 weeks also showed no adverse effects (Cragg et al. 1989).

In a series of studies in which Rhesus monkeys, rats, rabbits, and guinea pigs were exposed for 6-7 months to concentrations of ethylbenzene as high as 2,200 ppm, no toxic effects on lung histopathology were reported in any of the laboratory animals (Wolf et al. 1956). These parameters (i.e., toxic end points), however, were not well defined and may account for differing respiratory depression (RD$_{50}$) results reported in mice due to sensory irritation at 1,432 and 4,060 ppm by De Ceaurriz et al. (198 1) and Nielsen and Alarie (1982), respectively. The utility of the study by Wolf et al. (1956) is further limited by a general lack of study details (e.g., no exposure or control data were provided).

In the report of a go-day inhalation study, it was noted that both male and female Fischer 344/N rats exposed to 0, 99.4, 246,498,740, or 975 ppm ethylbenzene developed lung lesions and hyperplastic bronchiaVmediastina1 lymph nodes at doses of 246 ppm and above (NTP 1992). These effects were not seen in the exposed B6C3F$_1$ mice in the same study (NTP 1992). In addition, in the rats, increased relative lung weight was observed in males at 975 ppm, whereas female rats exhibited increased absolute and relative lung weight at 246 ppm ethylbenzene. It was the opinion of the NTP Pathology Working Group that "the pulmonary lesions and lymph node hyperplasia were more typical of an infectious agent than a response to the test compound." In the companion chronic-duration inhalation exposure of male and female Fischer 344 rats and B6C3F$_1$ mice to doses of 0, 75, 250, or 750 ppm for up to 2 years (103-104 weeks), no significant treatment-related histopathological effects were noted on respiratory tissue (NTP 1996).

Cardiovascular Effects. No adverse histopathological effects were reported for cardiac tissue in Fischer 344 rats or B6C3F$_1$ mice exposed to concentrations of ethylbenzene up to 782 ppm for 5 days a week, 6 hours a day for 4 weeks, or New Zealand White rabbits exposed to concentrations up to 1,610 ppm for 4 weeks (Cragg et al. 1989). In the report of a 90-day inhalation study, it was noted that neither male or female Fischer 344/N rats, nor B6C3F$_1$ mice exposed to 0, 99.4,246,498, 740, or 975 ppm ethylbenzene 5 days a week, 6 hours a day exhibited any adverse histopathological changes in cardiac tissue (NTP 1992). A similar lack of cardiovascular effects was noted in the companion chronic-duration inhalation exposure of male and female Fischer 344 rats and B6C3F$_1$ mice to doses of 0,75,250, or 750 ppm for up to 2 years (103-104 weeks) (NTP 1996).

A series of experiments using Rhesus monkeys, rats, rabbits, and guinea pigs exposed for 6-7 months to ethylbenzene concentrations ranging from 400 to 2,200 ppm re;orted no changes in the gross appearance of the heart or abnormal histopathological changes in cardiac tissue (Wolf et al. 1956). This study, however, is of little value because of a lack of study details (e.g., no cardiovascular data were presented), small number of study animals (e.g., one to two rabbits and monkeys), and poor definition of the parameters monitored.

Gastrointestinal Effects. In animals, a series of experiments using Fischer 344 rats and B6C3F$_1$ mice exposed to ethylbenzene concentrations as high as 782 ppm and New Zealand White rabbits exposed to ethylbenzene concentrations as high as 1,610 ppm for 4 weeks reported no changes in the gross appearance and no abnormal histopathological changes in the intestines (Cragg et al. 1989). In the report of a go-day inhalation study, it was noted that neither male or female Fischer 344/N rats, nor B6C3F$_1$ mice exposed to 0, 99.4,246,498,740, or 975 ppm ethylbenzene exhibited any adverse histopathological changes in gastrointestinal tissue, including cecum, colon, duodenum, esophagus, ileum, jejunum, and stomach (including forestomach and glandular stomach) (NTP 1992). No adverse histopathological effects were noted in the gastrointestinal tissues of male and female Fischer 344/N rats or B6C3F$_1$ mice exposed to concentrations up to 750 ppm for up to 2 years (NTP 1996).

Hematological Effects. Two studies involving long-term monitoring of workers occupationally exposed to ethylbenzene showed conflicting results with respect to effects on the hematopoietic system (Angerer and Wulf 1985; Bardodej and Cirek 1988). In one human study involving workers chronically exposed to organic solvents containing ethylbenzene, the average number of lymphocytes increased (p=0.05) and hemoglobin levels decreased (p=0.01l) in exposed individuals (n=35 males) compared with controls (Angerer and Wulf 1985). The average level of ethylbenzene in the blood of these workers at

which correlations were seen between exposure and hematological effects was 61.4 µg/L. Blood cell values of male workers examined in 1983 and 1984 showed average lymphocyte levels increased 68.8% and 41.5%, respectively, compared to controls. Average hemoglobin values decreased 7.1% and 5.2% in male workers examined in 1983 and 1984, respectively. Results showed that the hematopoetic system might be the target organ of a chronic exposure to alkylbenzene. However, whether the effect is due to one of the alkylbenzenes (e.g., ethylbenzene) or to the mixture was undetermined. Concomitant exposure to lead in these workers could be a confounding factor. The use of the median lead level, rather than the mean and variance of this measurement, could result in a lower estimate of the impact of concomitant exposure to lead. No adverse hematological effects were seen in a long-term study (20 years) on 200 male workers occupationally exposed to unspecified concentrations of ethylbenzene (Bardodej and Cirek 1988). Given the overall lack of a substantial amount of quantitative exposure data and simultaneous exposure to other hazardous chemicals such as xylene isomers, *n*-butanol, and C-9 aromatic hydrocarbons in the study of Angerer and Wulf (1985), these results are inadequate for evaluating hematological effects of ethylbenzene following inhalation exposure in humans.

Experiments with rats demonstrated a statistically significant increase in platelet counts in male Fischer 344 rats and a statistically significant increase in the mean total leukocyte count in female Fischer 344 rats exposed to 782 ppm ethylbenzene for 4 weeks (Cragg et al. 1989). Hematological parameters did not change for $B6C3F_1$ mice or New Zealand White rabbits exposed to the same or higher concentrations.

The effects of ethylbenzene on bone marrow counts and total blood counts were investigated in a series of experiments using Rhesus monkeys, rats, mice, rabbits, and guinea pigs exposed for 6-7 months to concentrations ranging from 400 to 2,200 ppm (Wolf et al. 1956). No effects were reported in any of the animals tested, but a number of limitations (e.g., small number of test animals) and poorly defined parameters (e.g., specific toxic end points that were investigated) may explain these results, which are different that those of Cragg et al. (1989). However, In a report of a 90-day inhalation study in Fischer 344/N rats and B6C3Fi mice, no adverse hematological effects were noted following exposure to ethylbenzene vapor concentrations of up to 975 ppm (NTP 1992). Based on the available data, no definitive conclusion can be drawn regarding the effect of ethylbenzene on hematological parameters.

Musculoskeletal Effects. Histopathological examination of bone tissue from Fischer 344 rats, B6C3F, mice, and New Zealand White rabbits exposed to concentrations of ethylbenzene up to 782 ppm in rats and mice, and 1,610 ppm in rabbits for 4 weeks revealed no bone tissue abnormalities in any of the

animals examined (Cragg et al. 1989). Neither was any adverse effect on bone tissue seen in
Fischer 344/N rats or B6C3F$_1$ mice exposed to 975 ppm ethylbenzene for 90 days, or 750 ppm for up to
2 years (NTP 1992, 1996). Given the limited data on musculoskeletal effects following ethylbenzene
inhalation exposure, no conclusions can be drawn.

Hepatic Effects. In a 20-year study of 200 male workers occupationally exposed to an undetermined
concentration of ethylbenzene, no cases of liver lesions or significant differences in liver function tests
between exposed and nonexposed workers were reported (Bardodej and Cirek 1988). During the entire
investigation period the risk of ethylbenzene exposure in this production plant was reported as negligible.

The results from several studies have suggested that hepatic effects may result from inhalation exposure to
ethylbenzene in laboratory animals. Studies with rats, mice, and rabbits showed differences in effects
across species (Andrew et al. 1981; Biodynamics 1986; Cragg et al. 1989; Elovaara et al. 1985, 1982;
Toftgard and Nilsen 1982; Wolf et al. 1956). No definitive conclusions can be drawn because limitations
are present in many of the studies. Effects include biochemical changes, histopathological alterations, and
an increase of liver weight relative to body weight. These changes may be an adaptive response, but
potential toxicity cannot be ruled out.

Hepatic congestion was observed upon microscopic examinations of liver tissue from Fischer 344 rats
exposed to 2,400 ppm for 4 days and from B6C3F$_1$ mice exposed to 1,200 ppm or 2,400 ppm for 4 days
(Biodynamics 1986; Cragg et al. 1989). Both groups of animals had died prior to termination of the
experiment. This liver congestion, however, may have been a secondary effect and may not be treatmentrelated.
No cause of death was reported for any of the animals. Biochemical changes (e.g., increases in
cytochrome P-450 concentration, NADPH-cytochrome reductase, 7-ethoxycoumarin 0-deethylase
[Toftgard and Nilsen 19821, and UDP glucuronyl-transferase) were reported in rats exposed to 2,000 ppm
ethylbenzene concentrations for 3 days and concentrations as low as 300 ppm for up to 16 weeks (Elovaara
et al. 1985). Electron microscopy also showed changes in hepatocyte ultrastructure (e.g., smooth
endoplasmic reticulum proliferation, slight degranulation of rough endoplasmic reticulum) in rats beginning
2 weeks after exposure to ethylbenzene (Elovaara et al. 1985). Fouchecourt and Riviere (1996) also
reported induction of hepatic enzymes (7-ethoxyresorufm 0-deethylase) in male and female Sprague-
Dawley rats and wild Norway rats exposed for up to 2 weeks in the laboratory to soil from a contaminated
petrochemical waste site that contained 0.2 ppm ethylbenzene among many other constituents. A slight
increase in hepatic catalase activity was also seen after 30-60 days of exposure to the contaminated soil.
In general, enzyme induction enhances the metabolism of ethylbenzene and may be considered an adaptive

phenomenon rather than a hepatotoxic effect. Increased liver-to-body-weight ratios were observed in male Sprague-Dawley rats exposed to 2,000 ppm ethylbenzene for 3 days (Toftgard and Nilsen 1982), Fischer 344 rats exposed to 400 ppm ethylbenzene for 4 days (Biodynamics 1986; Cragg et al. 1989), and Fischer 344 rats and B6C3F$_1$ mice exposed to 782 ppm ethylbenzene for 4 weeks (Cragg et al. 1989). Andrew et al. (1981) also reported increased relative liver weight in pregnant and nonpregnant Wistar rats and pregnant New Zealand White rabbits exposed to 959 and 962 ppm ethylbenzene, respectively, for 3 weeks prior to mating and throughout gestation. As with intracellular and biochemical changes, the significance of the increased relative liver weight with regard to possible health effects is unclear. No hepatic effects were observed in New Zealand White rabbits exposed to 2,400 ppm for 4 days, or 1,610 ppm for 4 weeks (Biodynamics 1986; Cragg et al. 1989).

Ethylbenzene exposure by inhalation for 6-7 months caused increased liver weights in Rhesus monkeys (600 ppm), rats (400 ppm), and guinea pigs (600 ppm), and histopathological changes including cloudy swelling in the liver of rats (2,200 ppm) (Wolf et al. 1956). No hepatic effects were reported in rabbits at 1,250 ppm (Wolf et al. 1956). The utility of this study, however, is limited by the lack of study details and statistical analysis of the histopathology results. Increased relative liver weights were also noted in a report of a 90-day inhalation study in which Fischer 344/N rats and B6C3F$_1$ mice were exposed to 249 ppm and 740 ppm ethylbenzene, respectively (NTP 1992). However, no corresponding organ dysfunction or histopathological change accompanied this observation. When Fischer 344/N rats and B6C3F$_1$ mice were exposed to concentrations of ethylbenzene up to 750 ppm for up to 2 years, male mice, but not rats, exhibited an increased incidence of syncytial alterations of the hepatocytes, hypertrophy, and hepatic necrosis; female mice exhibited an increased incidence of eosinophilic foci (NTP 1996).

Renal Effects. Renal effects, manifested as histopathological changes, enzymatic changes, or increased kidney-to-body-weight ratios, have been observed in a number of species following inhalation exposure to ethylbenzene (Andrew et al. 1981; Biodynamics 1986; Cragg et al. 1989; Elovaara et al. 1985; NTP 1992; Toftgard and Nilsen 1982; Wolf et al. 1956). The significance of these changes with regard to possible health effects is not known, but these studies suggest variations across species, indicating that rats and mice may be more susceptible to ethylbenzene-induced renal effects than rabbits, guinea pigs, and monkeys. However, this is difficult to determine given weaknesses (e.g., poor study details, lack of statistical analysis, small number of animals used) in many of these studies. Enzymatic changes in the kidney (e.g., increased concentration of 7-ethoxycoumarin, 0-deethylase, UDP glucuronyl-transferase, NADPHcytochrome c reductase) were reported in Sprague-Dawley rats following a 3-day exposure to 2,000 ppm

ethylbenzene (Toftgard and Nilsen 1982). Increased kidney-to-body-weight ratios were reported following a 4-day exposure of Fischer 344 rats to 1,200 ppm; and renal congestion was reported in Fischer 344 rats and B6C3F$_1$ mice following a 4-day exposure to 1,200 ppm ethylbenzene, but not in New Zealand White rabbits exposed to 2,400 ppm ethylbenzene for the same period of time (Biodynamics 1986; Cragg et al. 1989). These effects were not unusual in animals that died and were not exsanguinated. Therefore, this effect may be a secondary effect and may not be treatment-related.

Longer exposure durations produced renal effects at lower ethylbenzene exposure concentrations. Andrew et al. (1981) reported increased relative kidney weight in pregnant Wistar rats exposed to 959 ppm, followed by exposure to 985 ppm ethylbenzene, but no changes in pregnant New Zealand White rabbits exposed to 962 ppm ethylbenzene for 3 weeks prior to mating and throughout gestation. Dose-related increases in 7-ethoxycoumarin 0-deethylase, UDP glucuronyl-transferase and glutathione were reported in Wistar rats following a 5-16-week exposure to ethylbenzene at concentrations ranging from 50 to 600 ppm (Elovaara et al. 1985). In the same study, significant increases in the kidney-to-body-weight ratio were observed at weeks 2 and 9 in animals exposed to 400 ppm when compared with control animals. No renal changes were reported in Fischer 344 rats or B6C3F$_1$ mice exposed to ethylbenzene concentrations as high as 782 ppm for 4 weeks or in New Zealand White rabbits exposed to ethylbenzene concentrations as high as 1,610 ppm for the same duration (Cragg et al. 1989).

In rats, a slight increase in kidney weight was observed at 400 ppm, and swelling of the tubular epithelium in the kidney was observed in rats exposed to 600 ppm ethylbenzene for up to 7 months (Wolf et al. 1956). No toxic effects were reported in rabbits, guinea pigs or Rhesus monkeys. The usefulness of this study, however, is limited by the lack of study details and statistical analysis of the histopathology data. In a report of a 90-day inhalation study, it was noted that Fischer 344/N rats and B6C3F$_1$ mice exhibited increased relative kidney weights at ethylbenzene concentrations of 740 ppm and 975 ppm, respectively (NTP 1992). Regeneration of renal tubules in the kidneys of male rats was also seen in all exposure groups, including controls. It was noted that the degree of regeneration was somewhat greater in the 975 ppm group compared to the controls; however, this difference was not statistically significant. In the companion 2-year bioassay (NTP 1996), rats exhibited increased incidence of renal tubule hyperplasia at 750 ppm, but mice were not adversely affected.

Endocrine Effects. Three studies in animals addressed histopathological effects in endocrine organs after intermediate-duration inhalation exposure to ethylbenzene. Microscopic examination of the adrenals,

pancreas, pituitary, and thyroid/parathyroid glands from Fischer 344 rats, B6C3F$_1$ mice, and New Zealand White rabbits exposed to 782 ppm (rats and mice) or 1,610 ppm (rabbits) ethylbenzene for 6 hours a day, 5 days a week for 4 weeks showed no changes (Cragg et al. 1989). The NTP (1992) go-day study indicated no histopathological effect on the adrenal glands, pancreas, parathyroid glands, pituitary gland, or thyroid gland from Fischer 344/N rats and B6C3F$_1$ mice exposed to 975 ppm ethylbenzene. Wolf et al. (1956) evaluated the effects of inhalation exposure of Rhesus monkeys, rats, guinea pigs, and rabbits to ethylbenzene for 5 days a week, 7-8 hours a day for 6-7 months on adrenal and pancreatic tissue. No effect was seen in Rhesus monkeys at concentrations up to 600 ppm, in rats at 2,200 ppm, or in guinea pigs and rabbits at 1,250 ppm.

No adverse effect on endocrine glands was observed in rats exposed to concentrations of 75-750 ppm in the companion 2-year bioassay (NTP 1996). However, mice exposed to the same concentrations of ethylbenzene for 2 years exhibited an increased incidence of follicular cell hyperplasia in the thyroid gland at the high dose. In female mice exposed to 250 and 750 ppm ethylbenzene, the incidences of hyperplasia of the pituitary gland pars distalis were significantly greater than those in the control group.

Ocular Effects. Ethylbenzene concentrations of 1,000 ppm have been shown to cause momentary ocular irritation, a burning sensation, and profuse lacrimation in humans (Thienes and Haley 1972; Yant et al. 1930). These effects became more severe in humans exposed to 2,000 ppm ethylbenzene and intolerable at concentrations of 5,000 ppm or higher (Yant et al. 1930). The strength of these results, however, is diminished by a number of limitations (e.g., unclear exposure durations, impurities in ethylbenzene, and limited information on methodology for analysis of the concentrations used). Cometto-Muniz and Cain (1995) measured eye irritation in humans after exposure to ethylbenzene vapor. Eye irritation was observed at 10,000 ppm.

Similar ocular effects to those in humans were seen in animals exposed to ethylbenzene vapors. Eye irritation accompanied by tearing was observed in guinea pigs 8 minutes following exposure to 1,000 ppm ethylbenzene and 1 minute following exposure to 2,000-10,000 ppm ethylbenzene (Yant et al. 1930). Tegeris and Balster (1994) reported lacrimation and palpebral closure in male CFW mice after 20 minutes of exposure to 2,000 ppm ethylbenzene. After 4 days of inhalation exposure to 1,200 ppm ethylbenzene, Fischer 344 rats exhibited lacrimation (Biodynamics 1986; Cragg et al. 1989). B6C3F$_1$ mice and New Zealand White rabbits exhibited lacrimation after exposure to 400 ppm. After 4 weeks of exposure to 382 ppm, rats showed sporadic lacrimation, whereas mice and rabbits showed no ocular effects at 782 ppm

and 1,610 ppm, respectively (Cragg et al. 1989). No ocular effects were seen in Fischer 344/N rats and B6C3F$_1$ mice after a 13-week exposure to 975 ppm ethylbenzene (NTP 1992).

Ocular effects observed in humans and animals after inhalation exposure to ethylbenzene are presumed to be due to direct contact of the eyes with ethylbenzene vapor. These effects are discussed in Section 2.3.3.2.

Body Weight Effects. Studies in animals have addressed body weight effects of acute- and intermediate-duration inhalation exposure to ethylbenzene. After 4 days of exposure, Fischer 344 rats, B6C3F$_1$ mice, and New Zealand White rabbits showed no adverse effect on body weight at 1,200,400, and 2,400 ppm, respectively (Biodynamics 1986; Cragg et al. 1989). Similarly, Romanelli et al. (1986) saw no effect on body weight in New Zealand White rabbits after 7 days of exposure to 750 ppm.

Andrew et al. (1981) reported no change in body weight in pregnant Wistar rats or New Zealand White rabbits exposed to 985 and 962 ppm ethylbenzene, respectively, for 3 weeks prior to mating and throughout gestation. Elovaara et al. (1985) reported a decrease in body weight gain of 26-48% at weeks 2, 5, and 9, but not at week 16 in male Wistar rats exposed to 600 ppm ethylbenzene 6 hours a day, 5 days a week. No adverse effect on body weight was observed in Fischer 344 rats, B6C3F$_1$ mice, and New Zealand White rabbits exposed to 782 ppm (rats and mice) or 1,610 ppm (rabbits) ethylbenzene for 6 hours a day, 5 days a week for 4 weeks (Cragg et al. 1989). Wolf et al. (1956) evaluated the effects of inhalation exposure of Rhesus monkeys, rats, guinea pigs, and rabbits to ethylbenzene for 7-8 hours a day, 5 days a week for 6-7 months on body weight. No effect was seen in monkeys at concentrations up to 600 ppm, or in rats or rabbits at 1,250 ppm. Rats exposed to 2,200 ppm and guinea pigs exposed to 1,250 ppm showed some growth depression (data not shown). The NTP (1992) 90-day study indicated no effect on body weight in Fischer 344/N rats and B6C3F$_1$ mice exposed to 975 ppm ethylbenzene. Similarly, no significant effect on body weight was observed in the companion 2-year study in which rats and mice were exposed to concentrations of ethylbenzene of up to 750 ppm (NTP 1996).

Metabolic Effects. Biochemical changes in the liver and kidney (e.g., increases in microsomal protein content, NADPH-cytochrome reductase, 7-ethoxycoumarin 0-deethylase, and UDP glucuronyl-transferase) were reported in rats exposed to ethylbenzene concentrations as low as 300 ppm after 5 weeks of exposure, and at 600 ppm after 16 weeks (Elovaara et al. 1985). Electron microscopy also showed changes in hepatocyte ultrastructure (e.g., smooth endoplasmic reticulum proliferation, slight degranulation of rough endoplasmic reticulum) in rats beginning 2 weeks after exposure to ethylbenzene, indicating metabolic

activation of the liver. Increased enzyme activities in liver and kidney (cytochrome P-450 concentration, increased NADPH-cytochrome C reductase activity, 7-ethoxyresorufin, hydroxylation of *n*-hexane, and metabolism of benz[a]pyrene) were also observed in Sprague-Dawley rats exposed to 2,000 ppm ethylbenzene for 3 days (Toftgard and Nilsen 1982)

Other Systemic Effects. Fischer 344 rats exposed to 1,200 ppm ethylbenzene for 4 days exhibited yellow or brown anogenital staining (Biodynamics 1986; Cragg et al. 1989). Andrew et al. (1981) reported no change in food consumption in pregnant Wistar rats or New Zealand White rabbits exposed to 985 and 962 ppm ethylbenzene, respectively, for 3 weeks prior to mating and throughout gestation.

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were found regarding immunological effects in humans following inhalation exposure to ethylbenzene.

Andrew et al. (1981) reported increased relative spleen weight in pregnant Wistar rats, but not in pregnant New Zealand White rabbits exposed to 959 and 962 ppm ethylbenzene, respectively, for 3 weeks prior to mating and throughout gestation. Cragg et al. (1989) examined bone marrow (sternum), lymph nodes, thymic region, and spleen from Fischer 344 rats, B6C3F$_1$ mice, and New Zealand White rabbits exposed to 782 ppm (rats and mice) or 1,610 ppm (rabbits) for 4 weeks. No changes were seen in the tissues. Wolf et al. (1956) evaluated the effects on body weight of inhalation exposure of guinea pigs, and rabbits to ethylbenzene for 5 days a week, 7-8 hours a day for 6-7 months. No effect was seen the gross appearance of the spleen, or the bone marrow in guinea pigs or rabbits at 1,250 ppm. Both the NTP (1992) 90-day study and the NTP 2-year bioassay (NTP 1996) indicated no treatment-related effect on the histopathology of several tissues, including bronchial lymph nodes, regional lymph nodes, mandibular and mesenteric lymph nodes, mediastinal lymph nodes, spleen, or thymus in Fischer 344/N rats and B6C3F$_1$ mice exposed to 975 ppm ethylbenzene for 90 days or 750 ppm for 2 years.

The highest NOAELs for immunological and lymphoreticular effects in each species for intermediate- or chronic-duration exposure are reported in Table 2- 1 and plotted in Figure 2- 1.

2.2.1.4 Neurological Effects

Symptoms of dizziness accompanied by vertigo have been observed in humans acutely exposed to air concentrations of ethylbenzene ranging from 2,000 to 5,000 ppm (Yant et al. 1930). Complete recovery occurs if exposure is not prolonged. This study had a number of weaknesses (e.g., unclear exposure durations, impurities in ethylbenzene, and limited information on the methodology for analysis of vapor concentrations). No studies were found regarding neurological effects in humans following intermediate- or chronic-duration exposure.

The primary effects in animals following acute exposure to high air concentrations of ethylbenzene are neurological effects. Central nervous system depression and ataxia were observed in guinea pigs exposed to 2,000 ppm ethylbenzene for acute-duration periods (Yant et al. 1930). Moderate activation in motor behavior was observed in CFY rats following a 4-hour inhalation exposure to levels of ethylbenzene ranging from 400 to 1,500 ppm (Molnar et al. 1986). In the same study, narcotic effects were observed in CFY rats at ethylbenzene concentrations from 2,180 to 5,000 ppm. This study is limited by a lack of methodological detail and appropriate statistical analysis. In addition, Nielsen and Alarie (1982) were unable to determine whether respiratory effects observed in Swiss-Webster mice exposed via intratracheal instillation to various concentrations of ethylbenzene up to 7,800 ppm or 9,640 ppm by inhalation for a duration of 30 minutes were due to sensory irritation of the upper respiratory tract or central nervous system effects. Tegeris and Balster (1994) evaluated the neurobehavioral effects of ethylbenzene in adult male CFW (Charles River Swiss) albino mice exposed to 0, 2,000, 4,000, or 8,000 ppm for 20 minutes. Ethylbenzene at 2,000, 4,000, and 8,000 ppm produced changes in posture; decreased arousal and rearing; increased ease of handling; disturbances of gait, mobility, and righting reflex; decreased forelimb grip strength; increased landing foot splay; and impaired psychomotor coordination. These acute effects were short-lived and more pronounced during exposure than after exposure, with recovery beginning within minutes of removal from the exposure chamber. Sensorimotor reactivity also decreased. Salivation, prostration, and/or reduced activity were observed in Fischer 344 rats and B6C3F$_1$ mice exposed to 2,400 or 1,200 ppm ethylbenzene, respectively, for 4 days (Biodynamics 1986; Cragg et al. 1989). However, New Zealand White rabbits exposed to 2,400 ppm ethylbenzene for the same period of time showed no adverse behavioral effects. In addition, no significant dose-related behavioral changes and no histopathological alterations were reported in rats or mice exposed to concentrations of up to 782 ppm for 6 hours a day, 5 days a week for 4 weeks, although sporadic salivation was noted in rats at doses of 382 ppm and above (Cragg et al. 1989). Similarly, no behavioral changes or histopathological alterations

were observed in rabbits exposed to concentrations up to 1,610 ppm ethylbenzene for 4 weeks (Cragg et al. 1989). Changes in dopamine and other biochemical alterations were observed in Sprague-Dawley rats (Andersson et al. 1981) and New Zealand White rabbits (Mutti et al. 1988; Romanelli et al. 1986) exposed for 3-7 days to ethylbenzene concentrations of 2,000 and 750 ppm, respectively. Frantik et al. (1994) studied acute neurotoxicity of ethylbenzene in Wistar rats and H strain mice. Exposure to 245 and 342 ppm, respectively, resulted in a 30% depression of evoked electrical activity in the brain immediately after exposure. In a 90-day study (NTP 1992), Fischer 344/N rats and B6C3F$_1$ mice showed no adverse histopathological effects on brain tissue at doses up to 975 ppm. Similarly, no adverse effects were noted in the brain tissues of rats and mice exposed to concentrations of ethylbenzene of up to 750 ppm in the companion 2-year bioassay (NTP 1996). Differences in results in the studies using rats (Andersson et al. 1981; Molnar et al. 1986) and rabbits (Mutti et al. 1988; Romanelli et al. 1986) exposed to ethylbenzene are probably due to parameters monitored, duration of exposure, and analytical technique. Differences in results can also be attributed to differences in species studied (Biodynamics 1986; Cragg et al. 1989).

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 2- 1 and plotted in Figure 2- 1.

2.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following inhalation exposure to ethylbenzene.

No reproduction studies over one or more generation of animals were located for inhalation exposure to ethylbenzene. After acute exposure to concentrations as high as 2,400 ppm ethylbenzene for 4 days, no histopathological changes were noted in the testes of Fischer 344 rats, B6C3F$_1$ mice, or New Zealand White rabbits (Biodynamics 1986; Cragg et al. 1989). Ungvary and Tatrai (1985) evaluated the embryotoxic effects of benzene and its alkyl derivatives in CFY rats, CFLP mice, and New Zealand White rabbits. Pregnant rats, mice, and rabbits were exposed by inhalation to concentrations up to 553, 115, and 230 ppm respectively, continuously during organogenesis. Rats exhibited increased postimplantation death in all treated groups 2138 ppm, whereas mice exhibited no adverse effects. Rabbits exhibited increased abortions at the high dose.

No testicular histopathological abnormalities were reported in Fischer 344 rats and B6C3F$_1$ mice exposed to concentrations as high as 782 ppm and New Zealand white rabbits exposed to ethylbenzene concentrations as high as 1,610 ppm for 4 weeks (Cragg et al. 1989). Pre-gestational exposure for 3 weeks and gestational exposure of female Wistar rats and New Zealand White rabbits to concentrations of approximately 100 or 1,000 ppm ethylbenzene resulted in no conclusive evidence of reproductive effects in either species (Andrew et al. 1981).

NTP (1992) reported no effect on sperm or testicular morphology, or the length of the estrous cycle in Fischer 344/N rats or B6C3F$_1$ mice exposed to 975 ppm ethylbenzene for 90 days. For rats in the highdose group, the decrease in epididymal weight was not considered biologically significant since spermatid counts, sperm motility, and caudal weight were normal. Inhalation exposure of male Rhesus monkeys and rabbits to 600 ppm ethylbenzene for 6 months produced degeneration of germinal epithelium in the testes of one monkey and one rabbit (Wolf et al. 1956). Because there was only one male per exposure group, and because insufficient details on the study protocol were provided, the usefulness of this study is limited. No adverse histopathological effects were seen in the testes of rats or guinea pigs exposed to concentrations up to 1,250 or 600 ppm, respectively, for 6-7 months. Based on this limited evidence, no conclusions can be drawn concerning the possible reproductive consequence of this effect in animals.

In the NTP-sponsored 2-year bioassay, the incidence of interstitial cell adenoma in male Fischer 344/N rats exposed to 750 ppm was significantly greater than in the control group and slightly exceeded the historical control range for inhalation studies (NTP 1996). The incidence of bilateral testicular adenoma was also significantly increased in males exposed to 750 ppm. Adenoma in the testes was observed in 36 of 50, 33 of 50, 40 of 50, and 44 of 50 male rats exposed to 0, 75, 250, and 750 ppm, respectively. No adverse effects on the reproductive tissues of male and female B6C3F$_1$ mice were observed (NTP 1996).

The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species for acute-, intermediate-, and chronic-duration are recorded in Table 2- 1 and plotted in Figure 2- 1.

2.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans following inhalation exposure to ethylbenzene.

The developmental effects of inhalation exposure to ethylbenzene have been studied in rats, mice, and rabbits (Andrew et al. 1981; Ungvary and Tatrai 1985). In rats, exposure during gestation to ethylbenzene for 24 hours a day for 9 days at doses ranging from 138 to 552 ppm resulted in fetal resorption and retardation of skeletal development in surviving fetuses (Ungvary and Tatrai 1985). Increased incidence of extra ribs and anomalies of the urinary tract were observed at the 552 ppm dose level. No effects were observed after exposure to 138 ppm for 6 hours a day for 9 days (Ungvary and Tatrai 1985). Maternal toxicity was reported to be moderate and dose-dependent, but no data were presented. Andrew et al. (1981) investigated the teratologic effects of ethylbenzene exposures to rats. Wistar rats were exposed to 0,100, or 1,000 ppm (average exposure chamber concentration measured at 0,97, or 959 ppm, respectively) ethyl-benzene for 7 hours/day, 5 days/week for 3 weeks. They were then mated and exposed to 0, 100, or 1,000 ppm (average exposure chamber concentration measured at 96 or 985 ppm, respectively) ethyl-benzene 7 hours/day, 7 days/week through Gd 19. The rats were then killed and examined at Gd 21. Litters were examined for the presence of external, visceral, and skeletal abnormalities, as well as incidence of growth retardation and intrauterine mortality. There was no maternal toxicity, as shown by no histo-pathological changes in the ovaries, lung, kidney, or liver, and there were no treatment-related effects on food consumption or body weight in the rats. However, relative liver and kidney weights were significantly increased in both groups exposed to 1,000 ppm during gestation compared to the pregnant control groups. Relative liver weights of non-pregnant rats were also elevated for the same high-level exposure groups. There were no significant increases in major malformations or minor anomalies in any of the exposed groups. Increased incidence of fetuses with extra ribs (p<0.05) was noted in A-L (0-100 ppm), A-H (0-1,000 ppm) and H-H (1,000-1,000 ppm) exposure groups while rudimentary rib incidence was elevated only in the A-H (0-1,000 ppm) group. When gestational exposure is considered for comparative purposes, only the high dosed groups had increased incidence of supernumerary ribs on the basis of percent of litters affected (69% for A-H [0-1,000 ppm] versus 5 1.6% for H-H [1,000-1,000 ppm]). The range for all of the air control and low dosed rats was 36.4-48.5%.

Mice exposed to 115 ppm ethylbenzene during gestation demonstrated an increased incidence of anomalies of the urinary tract (Ungvary and Tatrai 1985). The nature of the renal malformation was not characterized and no maternal toxicity was reported.

Reduction in the weight of female fetuses was reported in rabbits exposed to 115 ppm during gestation (Ungvary and Tatrai 1985) but not following longer exposure to higher doses (up to 1,000 ppm) (Andrew et al. 198 1). New Zealand rabbits were artificially inseminated and exposed to 0, 100, or 1,000 ppm

(average exposure chamber concentration measured at 99±9 or 962±76 ppm, respectively) ethylbenzene on Gd 1-24 for 7 hours/day and killed on Gd 30. There were no changes in food consumption or body weights of animals exposed for 24 days to 99 or 962 ppm ethylbenzene. Relative lung and kidney weights were unremarkable. At 962 ppm, absolute and relative liver weights of pregnant rabbits were higher than pregnant control groups. Mean relative weights of kidneys of all pregnant and nonpregnant does were unremarkable. Histological examination of the lungs of the rabbits showed no treatment-related changes. No apparent treatment-related cellular changes occurred in the kidneys. The livers had variable degrees of hepatocellular vocalization characterized by rough, nondiscrete vacuole edges, resembling glycogen deposits; the incidence of this change was evenly distributed among the control and exposed groups. No treatment-related effects were observed in fetal size, placental weight, or intrauterine growth retardation. There were no significant incidences of major malformations, minor anomalies, or common variants in fetal rabbits exposed in utero to ethylbenzene. Maternal toxicity, embryotoxicity, growth retardation, and teratogenicity were not observed in rabbits exposed to 100 or 1,000 ppm ethylbenzene in this study.

As stated above, a statistically significant increase in the incidence of fetuses with supernumerary ribs was observed in rats exposed to 959 ppm ethylbenzene during Gd 1-19 (Andrew et al. 1981). A statistically significant increase in this anomaly was also seen in rats exposed to 959 ppm for 7 hours daily, 5 days a week for 3 weeks just prior to mating followed by exposure during Gd 1-19. However, supernumerary ribs are a non-specific variation often observed in rodent fetuses. No supernumerary ribs were observed in the 97 ppm dose group. Based on this value of 97 ppm, an intermediate-duration inhalation MRL of 1.0 ppm was calculated, as described in the footnote to Table 2-l and in Appendix A.

The published version of the study by Ungvary and Tatrai (1985) has many deficiencies, including poor reporting of the experimental conditions used, absence of significant data concerning the test chemical and generation of the exposure environment, insufficient number of dose levels tested, and details of maternal and fetal observations, including abnormalities. In contrast, the study by Andrew et al. (1981), is well documented, and was conducted according to the testing standards of the U.S. Government that were in force at the time of the study. Even so, the effects seen, and on which the intermediate-duration inhalation MRL was based, were minimal, and may not be relevant to human health. Therefore, the potential for developmental effects following ethylbenzene exposure in humans cannot be ascertained.

The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and duration category are recorded in Table 2- 1 and plotted in Figure 2-l.

2.2.1.7 Genotoxic Effects

Only one study was found that discussed genotoxic effects in humans after inhalation exposure to a mixture of chemicals, including ethylbenzene. Holz et al. (1995) determined low-level exposure to ethylbenzene and its effect on peripheral lymphocytes in workers in a styrene production plant. Twenty-five exposed workers were compared with 25 non-exposed control employees working at the same company. The concentration of ethylbenzene for exposed workers determined from active air sampling at four different locations (oven house, production control, storage facility, and distillation area) ranged from 365 to 2,340 mg/m^3 (84-539 ppm). Measurements performed at the pump house showed ethylbenzene concentration levels >4,000 mg/m^3 (921 ppm) which exceeded the detection limit of the sampling device. Ethylbenzene concentration levels for control workers ranged from 145 to 290 mg/m^3 (33-67 ppm). Genotoxic monitoring was performed by nuclease P1-enhanced ^{32}P-postlabeling of DNA adducts in peripheral blood monocytes, and DNA single strand breaks, sister chromatid exchange, and micronuclei in lymphocytes. The content of kinetochores in the micronuclei was determined by immunofluorescence with specific antibodies from the serum of calcinosis-Raynaud's phenomenon-oesophageal dismobility-sclerodactyly-telangiectasia syndrome of scleroderma (CREST) patients. Metabolite concentrations in urine of exposed workers confirmed absorption of the ethylbenzene. No genotoxic effects related to exposure were detected by DNA adduct formation or DNA single strand breaks and sister chromatid exchange. Increased kinetochore positive micronuclei in peripheral lymphocytes were observed in the total exposed group (p=0.007), exposed smokers (p=0.045), and exposed non-smokers (p=0.035); the frequency of total micronuclei in peripheral lymphocytes was unchanged. Results from this study are inconclusive with regard to the genotoxic effects of ethylbenzene, since the workers were exposed to a mixture of styrene, ethylbenzene, benzene, toluene, and xylenes. In addition, the sample size of 25 exposed workers and 25 non-exposed controls was very small.

Evaluation of micronucleated erythrocytes from peripheral blood samples from male and female B6C3Fi mice exposed to concentrations of ethylbenzene of up to 750 ppm for 13 weeks showed no increase in the frequency of occurrence (NTP 1996). Other genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

No association has been found between the occurrence of cancer in humans and occupational exposure to ethylbenzene. Only one study was located that monitored the condition of 200 male workers chronically

exposed to ethylbenzene (Bardodej and Cirek 1988). No cases of malignancy in workers monitored for 20 years were reported. However, no conclusions can be drawn from this study because no quantitative exposure information was provided, and the length of time for which the workers were monitored for tumors was only 20 years, which is insufficient for detecting long-latency tumors in humans. No other studies were found regarding cancer effects in humans exposed to ethylbenzene by inhalation.

Information concerning the carcinogenicity of ethylbenzene in animals comes from a recently completed NTP-sponsored bioassay (NTP 1996). Male and female Fischer 344/N rats and B6C3F[1] mice were exposed to 0,75, 250, or 750 ppm ethylbenzene for up to 2 years. Pathological findings in male Fischer 344/N rats exposed to 750 ppm ethylbenzene showed incidences of renal tubule adenoma and adenoma or carcinoma (combined) significantly greater than incidences in the control group. An extended evaluation of the kidneys showed significant increases in incidences of renal tubule adenoma and renal tubule hyperplasia in both male and female rats exposed to 750 ppm ethylbenzene. In males exposed to 750 ppm, the incidence of renal tubule adenoma or carcinoma (combined) was significantly increased. The severity of nephropathy was increased in both male and female rats exposed to 750 ppm ethylbenzene. The incidence of interstitial cell adenoma in males exposed to 750 ppm was significantly greater than in control group and slightly exceeded the historical control range for inhalation studies. The incidence of bilateral testicular adenoma was also significantly increased in males exposed to 750 ppm. Adenoma in the testes was observed in 36 of 50, 33 of 50, 40 of 50, and 44 of 50 male rats exposed to 0, 75, 250, and 750 ppm, respectively. In mice, the incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) were significantly greater in males exposed to 750 ppm than in the controls but were within the NTP historical control range. The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly greater in female mice exposed to 750 ppm than in the control group but were within the historical control ranges. The draft report indicates that these results indicate clear evidence of carcinogenicity in male rats, and some evidence of carcinogenicity in female rats and male and female mice.

2.2.2 Oral Exposure

2.2.2.1 Death

No studies were located regarding death in humans following oral exposure to ethylbenzene. However, lethality has been observed in laboratory animals following ingestion of ethylbenzene. The oral LDSO

(lethal dose, 50% kill) for gavage administration of ethylbenzene to Carworth Wistar rats was reported to be approximately 4,769 mg/kg ethylbenzene (Smyth et al. 1962). No short-term studies using ethylbenzene administered in food or drinking water were located.

In another oral study with rats exposed to ethylbenzene, the LD_{50} was reported to be approximately 3,500 mg/kg ethylbenzene (Wolf et al. 1956). The usefulness of these data, however, was questionable since the methodology by which this value was derived was not reported.

An oral LD_{50} value for rats is recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

No studies were located describing respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, body weight, or metabolic effects in humans or gastrointestinal, musculoskeletal, endocrine, dermal, ocular, body weight, or metabolic effects in animals after oral exposure to ethylbenzene.

One oral intermediate-duration study in rats was found in the literature (Wolf et al. 1956). Systemic effects described in this study are discussed below. The highest NOAEL values and all reliable LOAEL values for systemic effects in this study are recorded in Table 2-2 and plotted in Figure 2-2.

Respiratory Effects. The only animal study available in which ethylbenzene alone was administered to the animals presented no data on adverse respiratory effects in female rats orally exposed to 13.6-680 mg/kg ethylbenzene by gavage for 6 months (Wolf et al. 1956). The only parameters monitored were gross necropsy and histopathological effects. The utility of this study is limited because of poor protocol description and because the data on respiratory effects were not presented.

Cardiovascular Effects. In a single study in animals, female rats were exposed to 13.6-680 mg/kg/body weight ethylbenzene via gavage for 6 months (Wolf et al. 1956). The only parameter monitored was histopathology of the cardiac tissue. However, these data were not presented in the study. Therefore, no conclusions can be drawn.

Table 2-2. Levels of Significant Exposure to Ethylbenzene - Oral

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE							
Death							
1	Rat (Carworth-Wistar)	once (G)				4769 M (LD$_{50}$)	Smyth et al. 1962
INTERMEDIATE EXPOSURE							
Systemic							
2	Rat	6 mo 5 d/wk 1 x/d (GO)	Hemato	680 F			Wolf et al. 1956
			Hepatic	136 F	408 F (increased liver weight; cloudy swelling of parenchymal liver cells)		
			Renal	136 F	408 F (increased kidney weight; cloudy swelling of kidney tubular epithelium)		

[a]The number corresponds to entries in Figure 2-2.

F = female; (G) = gavage; (GO) = gavage in oil; LD$_{50}$ = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level

Figure 2-2. Levels of Significant Exposure to Ethylbenzene - Oral

Acute (≤14 days)

Intermediate (15-364 days)

Systemic

(mg/kg/day)

Death

Hematological

Hepatic

Renal

10000

■ 1r

1000

○ 2r

◑ 2r

◑ 2r

100

○ 2r

○ 2r

10

	Key	
r rat ■ LD₅₀ (animals)	The number next
◑ LOAEL for less serious effects (animals)	to each point corresponds to
○ NOAEL (animals)	entries in Table 2-2.

1
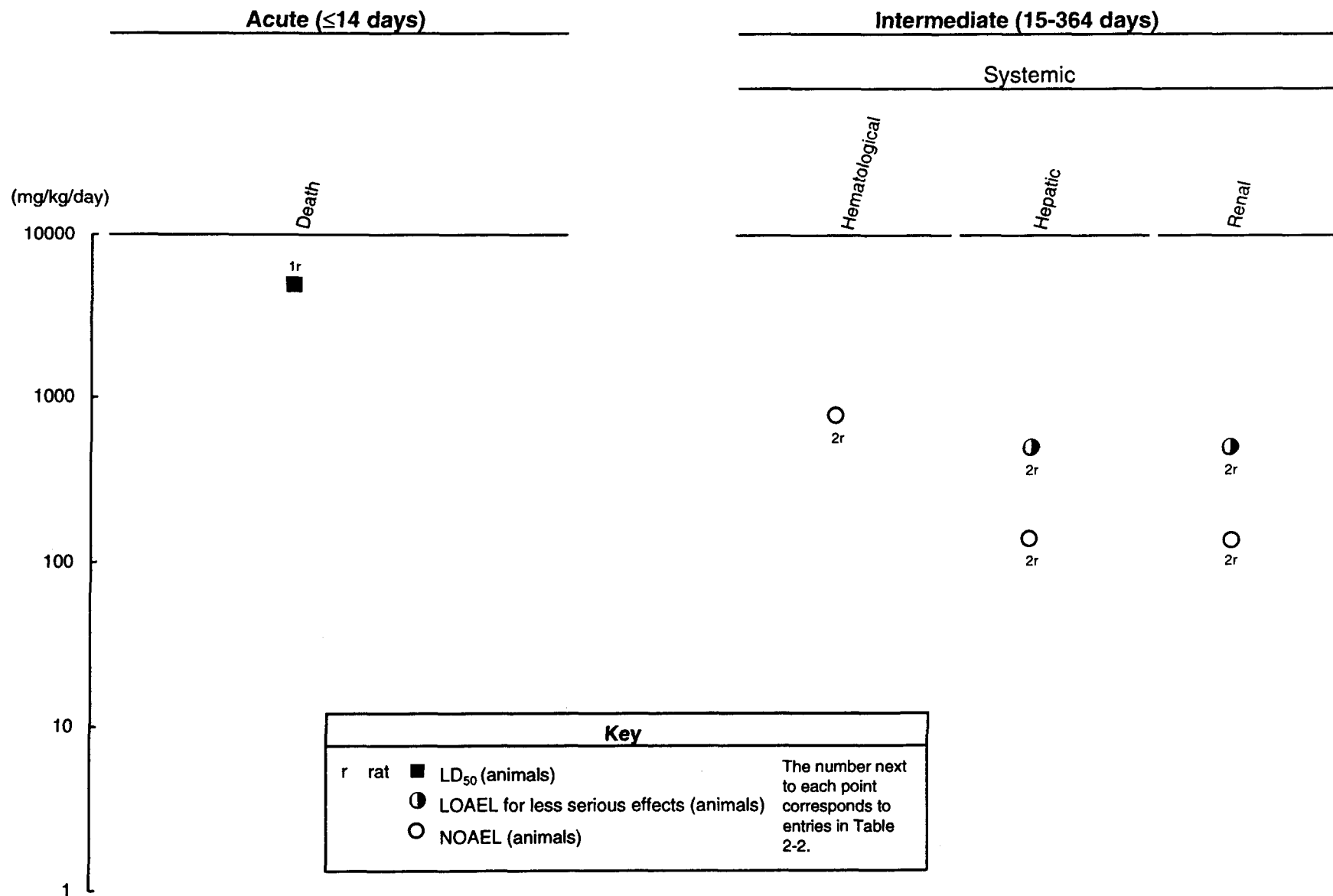
Hematological Effects. The only animal study available reported no adverse hematological effects in female rats orally exposed to 13.6-680 mg/kg/body weight ethylbenzene by gavage for 6 months (Wolf et al. 1956). The only parameters monitored, however, were bone marrow counts and total cell counts; thus, other hematological effects might have occurred but might not have been detected. Other weaknesses of this study include a poor description of study protocol and general lack of study details (e.g., hematological data).

Hepatic Effects. In the only animal study located, female rats were administered 13.6-680 mg/kg ethylbenzene by gavage for 6 months (Wolf et al. 1956). The authors reported histopathological changes characterized by cloudy swelling of parenchymal cells of the liver and an increase in liver weight in rats administered 408 mg/kg/day. No other hepatic changes were reported. No conclusions could be drawn from these results because of serious weaknesses in the methodology and reporting of the data (e.g., no data on number of animals with hepatic effects). Furthermore, no statistical analyses were performed.

Renal Effects. The only animal study that investigated renal effects following ethylbenzene exposure involved female rats administered 13.6-680 mg/kg/body weight ethylbenzene by gavage for 6 months (Wolf et al. 1956). Histopathological changes characterized as cloudy swelling of the tubular epithelium in the kidney and an increase in kidney weight were observed at the 408 mg/kg/day dose level. No other renal changes were reported. As in hepatic effects, no conclusions could be drawn from these results because of serious weaknesses in the methodology and reporting of the data (e.g., no data on the number of animals with renal effects). Furthermore, no statistical analysis were performed.

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals following oral exposure to ethylbenzene.

2.2.2.4 Neurological Effects
No studies were located regarding neurological effects in humans following oral exposure to ethylbenzene.

In an animal study that monitored behavioral changes, female rats were administered ethylbenzene by gavage for 6 months at concentrations ranging from 13.6 to 680 mg/kg/body weight (Wolf et al. 1956). No data on ethylbenzene-related behavioral changes were presented. No other parameters were investigated. The utility of this study is limited because the monitored behavioral changes were not reported, and the study protocol was poorly described. Given these weaknesses, no conclusions on neurological effects resulting from oral exposure to ethylbenzene can be drawn. No additional studies in animals were located regarding neurological effects following oral exposure to ethylbenzene.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following oral exposure to ethylbenzene.

The only available reproduction study with animals indicates that acute oral exposure to 500 or 1,000 mg/kg ethylbenzene decreases peripheral hormone levels and may block or delay the estrus cycle in female rats during the diestrus stage (Ungvary 1986). Decreased levels of hormones, including luteinizing hormone, progesterone, and 17 β-estradiol, were accompanied by uterine changes, which consisted of increased stromal tissue with dense collagen bundles and reduced lumen. No dose response was noted. The study limitations included lack of rationale for dose selection, use of only two doses, small number of test animals, and no statistical analysis of the data.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans and animals following oral exposure to ethylbenzene.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals following oral exposure to ethyl-benzene. Genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

No studies were located regarding carcinogenic effects in humans following oral exposure to ethylbenzene.

The carcinogenicity of ethylbenzene by the oral route has been evaluated in a chronic-duration study in Sprague-Dawley rats (Maltoni et al. 1985). A statistically significant increase in total malignant tumors was reported in females and in combined male and female groups exposed to 500 mg kg/day via gavage for 104 weeks and observed until after week 141. Evaluation of these results is difficult because no data on specific tumor type were presented. Other limitations of this study include the fact that only one dose was tested, and no information on survival was provided.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding lethal effects in humans following only dermal exposure to ethylbenzene. Matsumoto et al. (1992) reported the case of a 44-year-old man who was found unconscious in his gasoline vapor-filled car with his clothes wet with gasoline. The gasoline contained ethylbenzene among many other constituents. The patient emptied at least 18 L of gasoline into his car and was exposed to it for 10 hours or more. The patient died after 9 days of multiple organ failure.

The dermal LD_{50} in rabbits exposed to liquid ethylbenzene was calculated to be 15,433 mg/kg/body weight (Smyth et al. 1962). However, it is difficult to apply precise quantities of volatile compounds to the skin. No additional studies were located regarding death in animals following dermal exposure to ethylbenzene.

2.2.3.2 Systemic Effects

No studies were located regarding cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, body weight, or metabolic effects in humans or animals after dermal exposure to ethylbenzene.

The systemic effects observed after dermal exposure to ethylbenzene are discussed below. The highest NOAEL values and all reliable LOAEL values for each species and duration category are recorded in Table 2-3.

Respiratory Effects. Nose and throat irritation were reported in volunteers exposed to an ethylbenzene concentration of 2,000 ppm for 1-6 minutes (Yant et al. 1930). Three volunteers exposed to 5,000 ppm reported extreme irritation of the nose, and throat.

In animals, nasal irritation was reported in guinea pigs exposed to 1,000 ppm for 3 minutes, and in guinea pigs exposed to 2,000, 5,000, and 10,000 ppm for 480, 30, and 10 minutes, respectively (Yant et al. 1930). Gross histopathology revealed congestion and edema in the lungs with an increase in the severity of damage with increased exposure concentration (dose not specified).

Respiratory effects observed in humans and animals after inhalation exposure are assumed to be due to exposure of the mucous membranes and respiratory system to ethylbenzene vapor.

Dermal Effects. No studies were located regarding dermal effects in humans following dermal exposure to ethylbenzene. Matsumoto et al. (1992) reported the case of a 44-year-old man found unconscious in his gasoline vapor-filled car with his clothes wet with gasoline containing ethylbenzene among many other constituents. The patient emptied at least 18 L of gasoline into his car and was exposed to it for 10 hours or more. He was diagnosed as suffering from chemical burns and died after 9 days due to multiple organ failure.

Liquid ethylbenzene applied directly to the skin of an unspecified number of rabbits caused irritation characterized by reddening, exfoliation, and blistering (Wolf et al. 1956). Mild dermal irritation (grade 2 on a scale of 10) was also noted in New Zealand White rabbits 24 hours after application of ethylbenzene to clipped skin (Smyth et al. 1962).

Ocular Effects. Ocular effects observed in humans and animals after inhalation exposure are assumed to be due to exposure of the mucous membranes of the eye to ethylbenzene vapor. Volunteers reported eye irritation and burning, and profuse lacrimation which gradually decreased with continued exposure to 1,000 ppm for 1-6 minutes (Yant et al. 1930). Upon entering the chamber with an ethylbenzene concentration of 2,000 or 5,000 ppm, the volunteers also experienced severe eye irritation. Cometto-Muniz

Table 2-3. Levels of Significant Exposure to Ethylbenzene - Dermal

Species (Strain)	Exposure/ Duration/ Frequency	System	NOAEL	LOAEL		Reference
				Less serious	Serious	
ACUTE EXPOSURE						
Death						
Rabbit (New Zealand)	24 hr				15433 M (LD$_{50}$) mg/kg/d	Smyth et al. 1962
Systemic						
Rabbit (New Zealand)	24 hr	Dermal		8.67 mg (grade 4 skin irritation)		Smyth et al. 1962
INTERMEDIATE EXPOSURE						
Systemic						
Rat (F344/N)	13 wk 5 d/wk 6 hr/d	Ocular	975 ppm			NTP 1992
Mouse (B6C3F1)	13 wk 5 d/wk 6 hr/d	Ocular	975 ppm			NTP 1992

d = day(s); hr = hour(s); LD$_{50}$ = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; wk = week(s)

and Cain (1995) measured eye irritation in humans after exposure to ethylbenzene vapor. Eye irritation was observed at 10,000 ppm.

Liquid ethylbenzene applied directly to the eyes of rabbits for an unspecified duration caused slight irritation of conjunctival membranes (Wolf et al. 1956) and slight corneal injury (Smyth et al. 1962; Wolf et al. 1956).

Irritative effects from exposure to ethylbenzene vapor have been reported in animals. Tegeris and Balster (1994) reported lacrimation and palpebral closure in CFW mice after 20 minutes of exposure to 2,000 ppm ethylbenzene. Eye irritation was observed in guinea pigs exposed to 1,000 ppm for 8 minutes, and in animals exposed to 2,000, 5,000, and 10,000 ppm for 480,30, and 10 minutes, respectively (Yant et al. 1930). After 4 days of inhalation exposure to 1,200 ppm ethylbenzene, Fischer 344 rats exhibited lacrimation (Biodynamics 1986; Cragg et al. 1989). B6C3F$_1$ mice and New Zealand White rabbits exhibited lacrimation after exposure to 400 ppm. After 4 weeks of exposure to 382 ppm, rats showed sporadic lacrimation, whereas mice and showed no ocular effects at 782 and 1,610 ppm, respectively (Cragg et al. 1989). No ocular effects were seen in Fischer 344/N rats and B6C3F$_1$ mice after a 13-week exposure to 975 ppm ethylbenzene (NTP 1992).

No studies were located regarding the following health effects in humans or animals after dermal exposure to ethylbenzene:

2.2.3.3 Immunological and Lymphoreticular Effects
2.2.3.4 Neurological Effects
2.2.3.5 Reproductive Effects
2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

2.3 TOXICOKINETICS

The toxicokinetics of ethylbenzene have been examined in a number of studies. There is no information that suggests that ethylbenzene is handled differently by children than by adults. No specific information was found concerning ethylbenzene concentrations in breast milk, placenta, cord blood, or amniotic fluid. However, since ethylbenzene has been found in fat tissue, it is likely to be found in breast milk.

2.3.1 Absorption

2.3.1 .1 Inhalation Exposure

Inhalation studies in humans demonstrate that ethylbenzene is rapidly and efficiently absorbed via this route. Human volunteers exposed for 8 hours to ethylbenzene at concentrations of 23-85 ppm were shown to retain 64% of the inspired vapor, with only trace amounts detected in expired air (Bardodej and Bardodejova 1970). Another inhalation study that involved humans exposed to similar levels of ethylbenzene demonstrated mean retention rates of 49%, suggesting possible variability of absorption rates among individuals (Gromiec and Piotrowski 1984).

Matsumoto et al. (1992) determined the total gasoline concentration and that of several constitutive hydrocarbons in the blood of a 44-year-old man fatally exposed to the fumes from at least 18 L of gasoline for 10 hours or more. Blood samples were collected from 2 to 7 days and analyzed by gas chromatography/ mass spectrometry (GC/MS). The estimated initial concentration of ethylbenzene in the blood was 2.6 µg/mL with a half-life of 27.5 hours. This absorption value may have been slightly overestimated, however, because possible contributions from dermal exposure were not addressed.

Fustinoni et al. (1995) conducted environmental and biological monitoring of traffic policemen in Milan exposed to airborne aromatic hydrocarbons. Concentration values of ethylbenzene in air by passive diffusion personal samplers expressed as time-weighted average (TWA) in four subjects a day for 10 days showed mean outdoor concentration (n=20) of 37 mg/m^3 (range, 11-87 mg/m^3 or 2.5-20 ppm) as significantly higher than the mean indoor concentration (n=19) of 21 mg/m^3 (range, 2-40 mg/m^3 or 0.46-9.2 ppm). Blood concentrations of ethylbenzene found in non-smoking policemen before and after workshift showed no significant differences between outdoor (n=16; 158 ng/L before shift, 184 ng/L after shift) and indoor (n=14; 140 ng/L before shift, 162 ng/L after shift) groups and between before and after

workshift values for ethylbenzene for either indoor or outdoor exposure. No significant differences were found in blood concentrations obtained from non-smokers (n=30) and smokers (n=9) before (150 ng/L versus 197 ng/L) and after (174 ngL versus 222 ng/L) workshift.

Holz et al. (1995) determined low-level exposure to ethylbenzene and its effect on peripheral lymphocytes in workers in a styrene production plant. The concentration of ethylbenzene for exposed workers determined from active air sampling at four different locations (oven house, production control, storage facility, and distillation area) ranged from 365 to 2,340 $\mu g/m^3$ (84.1-538.9 pim). Measurements performed at the pump house showed ethylbenzene concentration levels >4,000 $\mu g/m^3$ (921 ppm), which exceeded the detection limit of the sampling device. Ethylbenzene concentration levels for control workers ranged from 145 to 290 $\mu g/m^3$ (33.4-66.8 ppm). Presence of metabolites in urine sampled after shift in the exposed workers indicated absorption of ethylbenzene had occurred.

In a study of chronic-duration exposure of factory workers at low levels of 2.1 ppm (geometric mean) or 2.3 ppm (arithmetic mean) ethylbenzene (maximum at 5 ppm), solvent concentrations in both whole-blood and serum samples collected at the end of shifts correlated significantly with the TWA concentrations of occupational exposure to ethylbenzene (Kawai et al. 1992).

Inhalation studies in animals exposed to ethylbenzene showed results similar to those found in humans. Harlan-Wistar rats rapidly absorbed radiolabeled ethylbenzene during respiration, with a retention rate of 44% (Chin et al. 1980b). This absorption value may have been slightly overestimated, however, because possible contributions from dermal exposure were not addressed. These studies did not correlate measured toxic effects with kinetic observations. No studies describing factors affecting absorption of ethylbenzene following inhalation exposure were available.

2.3.1.2 Oral Exposure

No studies were located regarding the absorption of ethylbenzene in humans following oral exposure. Studies in animals, however, indicate that ethylbenzene is quickly and effectively absorbed by this route. Recovery of ethylbenzene metabolites in the urine of rabbits administered a single dose of 593 mg/kg was between 72 and 92% of the administered dose 24 hours following exposure (El Masri et al. 1956). Similarly, 84% of the radioactivity from a single oral dose of 30 mg/kg ethylbenzene administered to female Wistar rats was recovered within 48 hours (Climie et al. 1983).

2.3.1.3 Dermal Exposure

Studies in humans dermally exposed to liquid ethylbenzene demonstrate rapid absorption through the skin, but absorption of ethylbenzene vapors through the skin appears to be minimal (Dutkiewicz and Tyras 1967; Gromiec and Piotrowski 1984). Absorption rates of 24-33 mg/cm^2/hour and 0.11-0.23 mg/cm^2/hour have been measured for male subjects exposed to liquid ethylbenzene and ethylbenzene from aqueous solutions, respectively (Dutkiewicz and Tyras 1967). The average amounts of ethylbenzene absorbed after volunteers immersed 1 hand for up to 2 hours in an aqueous solution of 112 or 156 mg/L ethylbenzene were 39.2 and 70.7 mg ethylbenzene, respectively. These results indicate that skin absorption could be a major route of uptake of liquid ethylbenzene or ethylbenzene in water. In contrast, ethylbenzene metabolite levels in urine following dermal exposure of human volunteers to ethylbenzene vapors did not differ from values taken prior to exposure, indicating minimal, if any, dermal absorption of ethylbenzene vapors (Gromiec and Piotrowski 1984).

Matsumoto et al. (1992) determined the total concentration of gasoline and several constitutive hydrocarbons in the blood of a 44-year-old man fatally exposed to the gasoline fumes and gasoline-soaked clothing and skin from at least 18 L of gasoline for 10 hours or more. Blood sample were collected from 2 to 7 days and analyzed by gas chromatography/mass spectrometry. The estimated initial concentration of ethylbenzene in the blood was 2.6 μg/mL with a half-life of 27.5 hours. The relative contributions of both inhalation and dermal absorption were not determined

The older, limited animal data on dermal absorption of liquid ethylbenzene are inconclusive. An unspecified number of rabbits dermally exposed to liquid ethylbenzene for 2-4 weeks demonstrated no apparent absorption through the skin (Wolf et al. 1956). However, this study is of limited value because absorption was measured only by overt signs of acute toxicity (e.g., gross appearance, behavior, and changes in body weight). The penetration rates of liquid ethylbenzene have been examined in excised rat skin (Tsuruta 1982). The penetration rates of ethylbenzene following 3-, 4-, and 5-hour exposure durations in rat skin were calculated to be 0.002,0.003, and 0.004 mg/cm^2/hour; these rates are substantially lower than the rate of dermal absorption determined for humans. This might be attributed to differences between *in vitro* and *in vivo* testing and/or differences in rat versus human skin.

Two more recent studies provide more definitive data on the *in vivo* absorption of ethylbenzene through rodent skin (Morgan et al. 1991; Susten et al. 1990). Morgan et al. (1991) investigated dermal absorption

of pure and dilute aqueous solutions of ethylbenzene in male Fischer 344 rats. Rats were exposed to either neat (99% purity), saturated (134 μg/mL), 2/3 saturated (84 μg/mL), or l/3 saturated (47 μg/mL) aqueous ethylbenzene for 24 hours. Blood samples were obtained from each rat via indwelling jugular catheters before addition of test chemical and after exposure for 0.5, 1, 2,4, 8, 12, and 24 hours. The concentration of ethylbenzene in the blood was determined by gas chromatography. Peak blood level during exposure to neat ethylbenzene was reported at 5.6 μg/mL attained after 1 hour of exposure, which decreased during the remainder of the exposure period. The concentration of ethylbenzene in the blood was highest after exposure to saturated aqueous solutions, followed by the 2/3 and 1/3 saturated solutions. The volume of ethylbenzene absorbed from the exposure cells after 24-hour dermal exposures was 0.24, 0.20,0.18, and 0.17 mL for neat, saturated, 2/3 saturated and 1/3 saturated, respectively. The volume of water absorbed by the animals in a 24-hour period when exposure cells contained only distilled water was 0.18 mL. This study suggests that in rats, significant amounts of ethylbenzene can be absorbed through the skin not only from the neat compound but also from an aqueous solution.

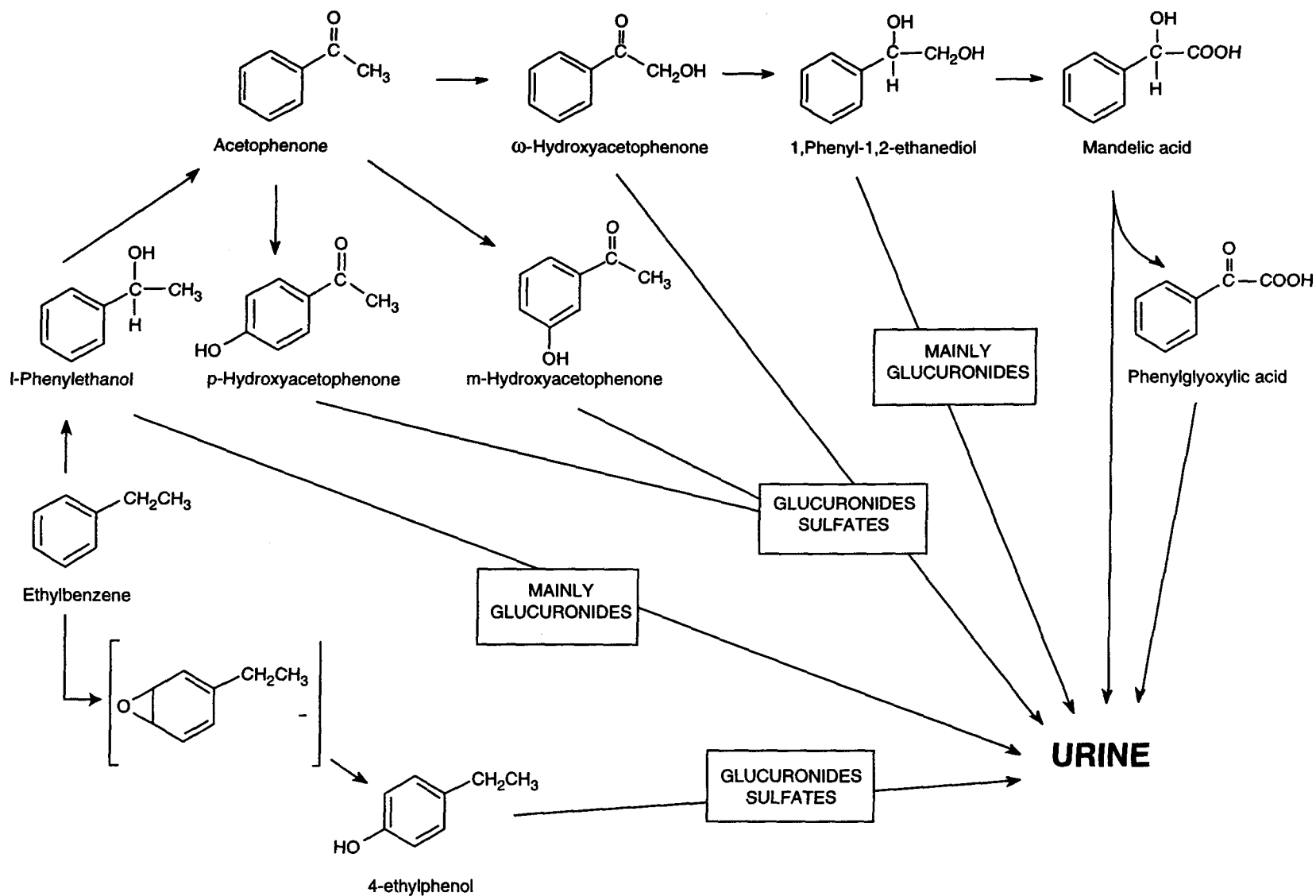Susten et al. (1990) conducted in viva percutaneous absorption studies of ethylbenzene in Hairless mice. Results showed total absorption (sums of radioactivity found in the excreta, carcass, skin application site, and expired breath) was 3.4% of the nominal dose. The total percentage recovered (includes wipe of skin area, ethylbenzene 0.03%) was 95.2%. The amount of ethylbenzene absorbed at an estimated contact time of 5 minutes was 148.55 μg with an absorption rate of 37 μg cmm^{-2} min^{-1}.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

In humans exposed for 2 hours to a mixture of industrial xylene containing 40.4% ethylbenzene, the estimated solvent retention in adipose tissue was 5% of the total uptake (Engstrom and Bjurstrom 1978). Since there was no indication of differences in turnover rates of chemicals within the mixture, it is likely that the retention of ethylbenzene in adipose tissue was approximately 2% of the total uptake. No studies were located concerning the distribution of ethylbenzene in humans following exposure to ethylbenzene alone. However, studies by Pierce et al. (1996) suggest that *in vitro,* the partitioning of ethylbenzene from air into human adipose tissue is similar to that observed in rats.

In rats, the concentrations of ethylbenzene in perirenal adipose tissue were reported to increase, although not linearly, with increasing concentrations of ethylbenzene (Engstrom et al. 1985) and in a mixture of solvent vapors containing ethylbenzene (Elovaara et al. 1982). The less-than-linear increase of ethylbenzene in adipose tissue with increasing dose was partially attributed to the induction of drug-metabolizing enzymes occurring with increasing exposure concentrations, altered blood flow to adipose tissue, changes in lung excretion, and changes in the distribution of ethylbenzene in different tissues. Ethylbenzene was shown to be efficiently distributed throughout the body in rats following inhalation exposure to radiolabeled ethylbenzene (Chin et al. 1980b). The highest amounts of radioactivity in tissues 42 hours after exposure to 230 ppm ethylbenzene for 6 hours were found in the carcass, liver, and gastrointestinal tract, with lower amounts detected in the adipose tissue.

2.3.2.2 Oral Exposure

No studies were located regarding distribution of ethylbenzene in humans following oral exposure. Data on the distribution of radiolabeled ethylbenzene hydroperoxide 1, 3, and 8 days after oral administration to rats were provided by Climie et al. (1983). Tissue residues were highest in the intestine, liver, kidney, and fat (0.53, 0.2, 0.21, and 0.27 μg/g tissue, respectively) 1 day after exposure and decreased to trace amounts (less than 0.05 μg/g tissue) in all tissues monitored (carcass, skin, muscle, and blood) 8 days after exposure. However, differences in the physical and chemical properties of ethylbenzene and ethylbenzene hydroperoxide (e.g., the potential of ethylbenzene hydroperoxide to generate free radicals) may affect distribution. No distribution data on radiolabeled ethylbenzene were provided.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution in humans following dermal exposure to ethylbenzene.

Susten et al. (1990) conducted *in vivo* percutaneous absorption studies of ethylbenzene in Hairless mice. Results showed that total absorption (sums of radioactivity found in the excreta, carcass, skin application site and expired breath) was 3.4% of the nominal dose. The percentages of absorbed doses following dermal application of [^{14}C]-ethylbenzene were: carcass, 15.5%; application site, 4.5%; expired breath, 14.3%; and excreta, 65.5%.

2.3.3 Metabolism

The metabolism of ethylbenzene has been studied in humans and other mammalian species. The data demonstrate that ethylbenzene is metabolized mainly through hydroxylation and then through conjugation reactions from which numerous metabolites have been isolated. Figure 2-3 summarizes the proposed metabolic pathway for ethylbenzene in humans (Engstrom et al. 1984). The major urinary metabolites have been identified (Kiese and Lenk 1974; Sullivan et al. 1976). Comparisons of in vitro data with data from intact animals indicate that liver microsomal enzymes may participate in ethylbenzene hydroxylation (McMahon and Sullivan 1966; McMahon et al. 1969), and evidence suggests that the adrenal cortex may be a major site of extra-hepatic ethylbenzene metabolism (Greiner et al. 1976). No significant differences in metabolism between oral and inhalation routes were reported in humans or animals. The metabolism of ethylbenzene has been found to vary with species, sex, and nutritional status. These differences are described below.

In humans exposed via inhalation, the major metabolites of ethylbenzene are mandelic acid (approximately 64-71%) and phenylglyoxylic acid (approximately 19-25 %) (Bardodej and Bardodejova 1970; Engstrom et al. 1984). Based on data from human, animal, and *in vitro* studies, the metabolic pathway for ethylbenzene in humans was proposed (Engstrom et al. 1984). This pathway is shown in Figure 2-3. Evidence indicates that the initial step in this metabolic pathway is oxidation (hydroxylation) of the side chain of ethylbenzene to produce 1-phenylethanol. Microsomal preparations from rat liver have shown that the oxidation of ethylbenzene proceeds with the incorporation of atmospheric oxygen, as opposed to oxygen from water molecules (McMahon et al. 1969). Flipovic et al. (1992) have shown that cytochrome P-450$_{(cam)}$ from *Pseudomonas putidu* provides a useful metabolic model for ethylbenzene hydroxylation, converting ethylbenzene to 1-phenylethanol at 98%. 1-Phenylethanol is conjugated to glucuronide, which then is either excreted or converted to subsequent metabolites. Oxidation of 1-phenylethanol yields aceto-phenone, which is both excreted in the urine as a minor metabolite and further transformed. Continued oxidation of the side chain leads to the sequential formation of 2-hydroxyacetophenone, 1 -phenyl- 1,2-eth-anediol mandelic acid, and phenylglyoxylic acid. Minor pathways (e.g., ring hydroxylation) include glucuronide and sulfate conjugation with hydroxylated derivatives to form glucuronides and sulfates that are excreted in the urine. Analysis of urine from humans exposed to ethylbenzene via the inhalation route showed that approximately 70 and 25% of the retained dose of ethylbenzene is excreted as mandelic acid and phenylglyoxylic acid, respectively (Bardodej and Bardodejova 1970; Engstrom et al. 1984). Additional

Figure 2-3. Metabolic Scheme for Ethylbenzene in Humans

metabolites detected in human urine include 1-phenylethanol(4%), p-hydroxyacetophenone (2.6%), m-hydroxyacetophenone (1.6%), and trace amounts of 1-phenyl- 1,2-ethanediol, acetophenone, 2-hydroxy-acetophenone, and 4-ethylphenol. Following dermal exposure of humans, however, excretion of mandelic acid was shown to be only 4.6% of the absorbed dose (Dutkiewicz and Tyras 1967), which may indicate differences in the metabolic fate between inhalation and dermal exposure routes. However, the small percentage of absorbed dose accounted for limits the interpretation. No animal data were located which could confirm these metabolic differences following dermal exposure. Generally, ethylbenzene metabolites and intermediates are thought to be only slightly toxic, since no adverse effects from human experimental exposure have been reported (Bardodej and Bardodejova 1970).

Qualitative and quantitative differences in the biotransformation of ethylbenzene in animals as compared to humans have been reported (Bakke and Scheline 1970; Climie et al. 1983; El Masri et al. 1956; Engstrom 1984; Engstrom et al. 1985; Smith et al. 1954a, 1954b; Sollenberg et al. 1985). The major metabolites of ethylbenzene differ from species to species, and different percentages of the metabolites are seen in different species. The principal metabolic pathway in rats is believed to begin with oxidation (hydroxylation) of the side chain as in humans (Climie et al. 1983; Engstrom 1984; Engstrom et al. 1985; Smith et al. 1954a). In rats exposed by inhalation or orally to ethylbenzene, the major metabolites were identified as hippuric and benzoic acids (approximately 38%), I-phenylethanol (approximately 25%), and mandelic acid (approximately 15-23%), with phenylglyoxylic acid making up only 10% of the metabolites (Climie et al. 1983; Engstrom 1984; Engstrom et al. 1985). Both *in vivo* studies using rats and *in vitro* studies using rat liver microsomes showed that 4-ethylphenol was also produced from ethylbenzene, perhaps by rearrangement of corresponding arene oxides (Bakke and Scheline 1970; Kaubisch et al. 1972). Kaubisch et al. (1972) also showed that 2-hydroxyethylbenzene was produced from ethylbenzene *in vitro* in the presence of rat liver microsomes. The level of ethylbenzene exposure was shown to affect the metabolic pattern. This was thought to be due either to selective enzymatic induction in the biotransformation of ethylbenzene or to delayed excretion of certain metabolites with increasing doses.

Further clarification of ethylbenzene metabolic pathways was provided by Sullivan et al. (1976). Using intraperitoneally dosed rats, the authors demonstrated that the conversion of 1-phenylethanol to mandelic acid initially involves oxidation to acetophenone. Acetophenone was considered to be the precursor of mandelic acid, benzoylformic acid, and benzoic acid. A similar study in which rabbits were intraperitoneally injected with a single dose of 250 mg ethylbenzene/kg body weight was conducted by Kiese and Lenk (1974). This study showed that between 1% and 10% of the dose was excreted as

1 -phenylethanol in the urine and less than 1% was excreted in the urine as 2-hydroxyacetophenone, *p*-hydroxyacetophenone, and *m*-hydroxyacetophenone.

Rabbits given an oral dose of ethylbenzene showed the major metabolic pathway to be hydroxylation of the a-carbon to 1-phenylethanol, which is oxidized further to a number of intermediates and metabolites (El Masri et al. 1956; Smith et al. 1954a). Many of these intermediates are subsequently conjugated to glucuronides and sulfates and excreted. In rabbits, the most important metabolite is hippuric acid, which is probably formed by oxidative decarboxylation of phenylglyoxylic acid (El Masri et al. 1958). Oxidation of the methyl group of ethylbenzene was also shown to occur, as evidenced by the presence of phenaceturic acid in the urine. A slight increase in the excretion of thioether suggests that glutathione conjugation may also play a minor role.

The nutritional status of animals was demonstrated to have a marked effect on ethylbenzene metabolism in rats (Nakajima and Sato 1979). The in vitro metabolic activity of liver microsomal enzymes on ethylbenzene was shown to be significantly enhanced in fasted rats despite a marked loss of liver weight. No significant increases in the microsomal protein and cytochrome P-450 contents were detected in fasted rats compared with fed rats. In addition, the metabolic rate in fasted males was significantly higher than in fasted females, but the difference in rates decreased following food deprivation for 3 days. These results suggest possible sex differences in the rate of ethylbenzene metabolism. However, it is not known if such differences exist in the normally fed rats.

Metabolism of ethylbenzene has not been studied in children or immature animals. However, some members of two of the enzyme superfamilies involved in conjugation of phase I ethylbenzene metabolites are known to be developmentally regulated. In humans, UDP glucuronosyltransferase activity does not reach adult levels until about 6-18 months of age, although the development of this activity is isoform specific. Activity of sulfotransferases seems to develop earlier, although again, it is isoform specific. The activity of some sulfotransferase isoforms may even be greater during infancy and early childhood than in adults (Leeder and Kearns 1997).

2.3.4 Elimination and Excretion

2.3.4.1 Inhalation Exposure

Excretion of ethylbenzene has been studied in humans and in a number of animal species. Ethylbenzene has been shown to be rapidly metabolized and then eliminated from the body, primarily as urinary metabolites. The major metabolic products have been previously described in Section 2.3.3. Elimination of ethylbenzene has been studied in human volunteers exposed by inhalation (Bardodej and Bardodejova 1970; Dutkiewicz and Tyras 1967; Engstrom and Bjurstrom 1978; Gromiec and Piotrowski 1984; Yamasaki 1984), or humans exposed by inhalation in the occupational setting (Holz et al. 1995; Kawai et al. 1991, 1992; Ogata and Taguchi 1988). The elimination of the ethylbenzene metabolite, mandelic acid, was reported to be rapid, with the acid detected in the first urine sample following the initiation of an g-hour inhalation exposure to 0, 4, 8, 18, 35, or 46 ppm ethylbenzene (Gromiec and Piotrowski 1984). Elimination of mandelic acid was reported to be biphasic, with half-lives of 3.1 hours for the rapid phase and 25 hours for the slow phase (Gromiec and Piotrowski 1984). During the g-hour exposure, 23% of the retained ethylbenzene was eliminated in the urine, and 14 hours following termination of exposure an additional 44% of the retained ethylbenzene was eliminated. The highest excretion rate of urinary metabolites in humans exposed to ethylbenzene by inhalation occurred 6-10 hours after the beginning of exposure (Gromiec and Piotrowski 1984; Yamasaki 1984). The metabolic efficiency was reported to be independent of the exposure dose.

Occupational studies have also been conducted to determine excretion and elimination of ethylbenzene and its metabolites after inhalation exposure. Holz et al. (1995) determined low-level exposure to ethylbenzene and its effect on peripheral lymphocytes in workers in a styrene production plant. The concentration of ethylbenzene for exposed workers determined from active air sampling at four different locations (oven house, production control, storage facility, and distillation area) ranged from 365 to 2,340 $\mu g/m^3$ (84-539 ppm). Measurements performed at the pump house showed ethylbenzene concentration levels >4,000 $\mu g/m^3$ (921 ppm) which exceeded the detection limit of the sampling device. Ethylbenzene concentration levels for control workers ranged from 145 to 290 $\mu g/m^3$ (33.4-66.8 ppm). Metabolite concentrations in urine sampled after shift in the exposed workers showed significantly higher exposure to ethylbenzene (mandelic acid, 43.9 mg/g creatinine p<0.001; phenylglyoxylic acid, 22.3 mg/g creatinine <0.05) compared to after-shift urine samples from non-exposed workers (mandelic acid, 4.3 mg/g

creatinine; phenylglyoxylic acid, 0.5 mg/g creatinine). Urine samples compared before shift showed significant exposure of workers to ethylbenzene (mandelic acid, 13.3 mg/g creatinine p<0.001; phenylglyoxylic acid, 10.7 mg/g creatinine p=0.006) compared to controls (mandelic acid, 5.5 mg/g creatinine; phenylglyoxylic acid, 2.8 mg/g creatinine).

Kawai et al. (199 1) determined urinary metabolites after exposure of metal workers to a solvent mixture containing toluene, mixed xylenes, and ethylbenzene. The employees worked on dip-coated metal parts with rust-resistant paint in which technical grade xylene mixture was used as the sole solvent. The workers wore protective gloves but did not wear masks. Vapor exposure from the solvent mixture contained mixed xylene, toluene, and ethylbenzene. The TWA concentration of ethylbenzene in breathing-zone air of the 121 workers during the shift was 0.9 ppm (geometric mean vapor concentration); maximum value observed was 11.4 ppm. The excretion of ethylbenzene metabolites, mandelic acid and phenylglyoxylic acid, showed geometric mean concentrations of 13.7 (maximum 52 ppm) and 8 ppm (maximum 128 ppm), respectively. The correlation of ethylbenzene exposure with phenylglyoxylic acid excretion was statistically insignificant (p>0.005). The correlation of ethylbenzene exposure with mandelic acid excretion was significant (p<0.001); both the correlation coefficient and the slope of the regression line were small. Kawai et al. (1991) reported that technical difficulties were encountered in the analysis of urine for mandelic acid and that low ethylbenzene exposure made accurate quantification difficult. In a study of chronic-duration exposure at lower levels of 2.1 ppm (geometric mean) or 2.3 ppm (arithmetic mean) with a maximum at 5 ppm, no significant correlation was observed in the relation of ethylbenzene with its metabolites, phenylglyoxylic acid and mandelic acid (Kawai et al. 1992).

Ogata and Taguchi (1988) determined urinary metabolites after toluene, ethylbenzene, and mixed xylene exposure of paint-factory workers. The recoveries of mandelic acid, hippuric acid, o-methylhippuric acid (o-MHA), m-methylhippuric acid (m-MHA) and creatinine added to urine (n=5) ranged from 100 to 101.6, 98.9-100.6, 99-99.9, 97.2-99.9, and 98-99.3%, respectively, in exposed workers. No mandelic acid or MHAs were detected in the urine of 32 unexposed subjects examined.

In animals, elimination of ethylbenzene metabolites following inhalation exposure is rapid and occurs primarily via urinary metabolites (Chin et al. 1980a, 1980b; Engstrom 1984; Engstrom et al. 1985) and to a much lesser degree via the feces and expired carbon dioxide (Chin et al. 1980b). Rats exposed to 230 ppm radiolabeled ethylbenzene for 6 hours via inhalation excreted virtually all of the radioactivity within 24 hours after the onset of exposure (Chin et al. 1980a, 1980b). Ninety-one percent of the

radioactivity was recovered, primarily in the form of urinary metabolites. In a similar inhalation experiment using rats exposed to 300 or 600 ppm, urinary excretion was reported to be 83% and 59% of the absorbed dose within 48 hours after the onset of exposure, with 13% eliminated during the first 6 hours of exposure (Engstrom 1984).

Quantitative differences between species in the percentages of metabolites excreted in the urine were also reported by Chin et al. (1980a). In this report, urinary metabolites in dogs and rats exposed to ethylbenzene by inhalation were studied. Although similarities in the types of metabolites recovered following inhalation exposure were reported, quantitative differences, albeit minor ones, were noted in the ratio of metabolites present in the urine. These results were attributed to differences in metabolism between dogs and rats.

2.3.4.2 Oral Exposure

No studies were located regarding the excretion of ethylbenzene metabolites in humans following oral exposure to ethylbenzene.

Elimination of ethylbenzene and its metabolites in animals after oral exposure has been shown to be similar to that following inhalation exposure. Female rats administered a single oral dose of 30 mg radiolabeled ethylbenzene/kg/body weight showed very rapid elimination, mostly in the urine (Climie et al. 1983). Eighty-two percent of the radioactivity was detected in the urine, while 1.5% was detected in the feces. The major metabolites were mandelic acid (23%) and hippuric acid (34%), with 1-phenylethyl glucuronide detected as a minor metabolite. Relatively minor metabolites (e.g., 4-ethylphenol, 2-phenylethanol, 1-phenylethanol) were shown to be excreted in the urine of male rats exposed to a single oral dose of 100 mg/kg ethylbenzene administered by gavage in oil (Bakke and Scheline 1970). No data on the major metabolites were provided in this study.

In a similar study in which male rats were given single oral doses of 350 mg/kg/body weight ethylbenzene, the excretion of mandelic acid and phenylglyoxylic acid was detected in the first urine sample after exposures. Peak concentration was reached within 17 hours, and ethylbenzene was virtually eliminated 48 hours following the onset of exposure (Sollenberg et al. 1985).

As in inhalation experiments, quantitative and qualitative differences between species were shown to exist in the percentages of metabolites excreted in the urine. Rabbits orally exposed to ethylbenzene excreted large amounts of glucuronide conjugates in the urine (El Masri et al. 1956; Smith et al. 1954a, 1954b) instead of mandelic acid, hippuric acid, and phenylglyoxylic acid, which are the major metabolites in rats (see above). Glucuronide conjugates accounted for 32% of the administered dose, with mandelic acid making up only 2% of the administered dose (El Masri et al. 1956). These results were confirmed in a study by Smith et al. (1954a, 1954b), who detected 32% of a single oral dose of ethylbenzene (433 mg/kg) administered to rabbits as glucuronide conjugates excreted in the urine.

2.3.4.3 Dermal Exposure

In humans, the pattern of excretion of ethylbenzene metabolite following dermal exposure has been shown to differ significantly from the pattern in which humans have been exposed by inhalation. Excretion of mandelic acid in humans dermally exposed to ethylbenzene was only 4.6% of the absorbed ethylbenzene (Dutkiewicz and Tyras 1967). Interpretation is difficult due to the small percentage of absorbed dose accounted for. No ethylbenzene was reported to be excreted in exhaled air. No further details on the excretion patterns were provided.

Susten et al. (1990) conducted *in vivo* percutaneous absorption studies of ethylbenzene in Hairless mice. Results showed total absorption (sums of radioactivity found in the excreta, carcass, skin application site, and expired breath) was 3.4% of the nominal dose. The absorbed doses collected in expired breath during the first 15 minutes of ethylbenzene application was 9.3%. The percentage of absorbed doses following dermal application of [^{14}C]-ethylbenzene are as follows: in the carcass, 15.5%; in the application site, 4.5%; expired breath, 14.3%; and excreta, 65.5%.

2.3.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based

pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites)

based on the results of studies where doses were higher or were administered in different species. Figure 2-4 shows a conceptualized representation of a PBPK model.

If PBPK models for ethylbenzene exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

The only PBPK model for ethylbenzene found in the literature is discussed below.

2.3.5.1 Summary of PBPK Models.

One PBPK model for ethylbenzene was found in the literature (Shatkin and Brown 1991). The model describes the pharmacokinetics of the dermal route of exposure to ethylbenzene in aqueous solution. The model was able to predict 94% of experimental data in humans under the same conditions.. Comparisons of dermal absorption versus inhalation and ingestion were made. This model contained no factors for children, fetuses, pregnant women, infants, or lactating women.

2.3.5.2 Ethylbenzene PBPK Model Comparison.

Since only one model was found, no comparisons can be made.

2.3.5.3 Discussion of Models.

The Shatkin and Brown Model.

Shatkin and Brown (1991) described a kinetic model of dermal absorption of several nonpolar organic nonelectrolytes in dilute aqueous solution, one of which was ethylbenzene.

Risk assessment. The authors state that the model has potentially useful applications for risk assessment if used within its limitations. The model was able to predict 94% of the experimental results with humans under the same conditions. In the case of ethylbenzene, the prediction was accurate enough to allow the authors to suggest that dermal absorption of some volatile organic chemicals from aqueous

Figure 2-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance
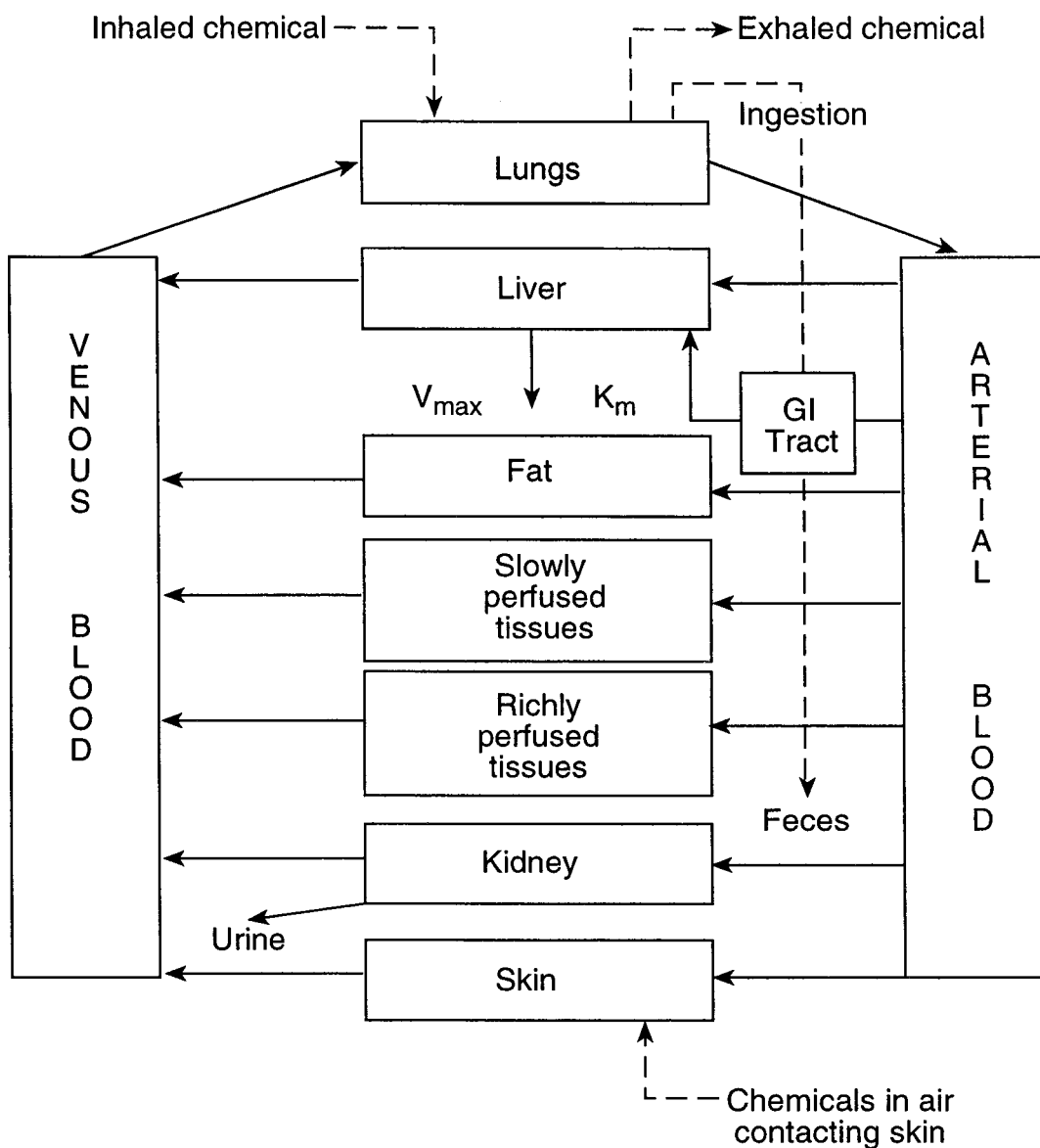
Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

solutions may be significant, and that the risk of such exposure should receive attention from the regulatory agencies. The predictions made for ethylbenzene absorption with the Shatkin and Brown (1991) model suggest that the model may be useful in estimating absorption of this compound from aqueous media through bathing, swimming, and other activities that may bring people in contact with ethylbenzenecontaminated

water.

Description of the model. Shatkin and Brown (1991) presented their model both with a traditional compartmental diagram, and a scheme indicating the physiological significance of the model (Figure 2-5). Three body compartments were represented in the traditional model: stratum corneum, viable epidermis, and blood. Molecules of the solvent were assumed to diffuse through fully hydrated stratum corneum and viable epidermis in a dissolved state by purely passive means, with passage through the stratum corneum being the rate-limiting step. A uniform thickness of 40 μm was assumed for the stratum corneum, with adjustments for different body parts. Immersion of the hand, or of the full body was assumed for the predicted models. The viable epidermis was assumed to be 200 μm, although the thickness was varied to test the outcome of the model.

Blood was the third kinetic compartment used in the traditional model. No distinction was made between blood in the dermal circulation and blood in the systemic circulation. Transfer of the chemical from the epidermis into the blood was considered to be proportional to the amount of chemical in the epidermis, the epidermal blood flow, and the relative solubility of the chemical in the two compartments (epidermis/blood partition coefficient). Blood and epidermis were considered to be in equilibrium. The change in the amount of chemical in the epidermis was considered to be dependent upon the rate of entry from, and removal to the stratum comeum, removal into the blood, and reentry from the blood.

The overall elimination rate of the chemical from the blood includes excretion via inhalation, urinary filtration, metabolism, and disposition in other body compartments. First-order kinetic behavior was assumed for all compartments.

The model was conceptualized based on physiological relationships, as shown in Figure 2-5. The model was then entered into a computer program, where the conceptual relationships between the compartments defined in Figure 2-5 are described mathematically with parameters shown in Table 2-4.

Figure 2-5. Schematic Representation of the Model of Dermal Absorption

bath to
SC rate

SC to
epidermis rate

epidermis to
blood rate

bathtub

blood

excreted

stratum corneum

epidermis

elimination rate

SC to bath rate

epidermis to SC rate

blood to epidermis rate

outer
reservoir

stratum
corneum

viable
epidermis

blood

urine
exhaled breath
organ storage
metabolism
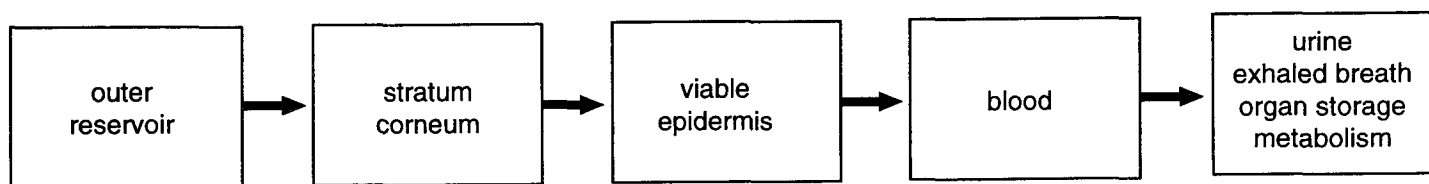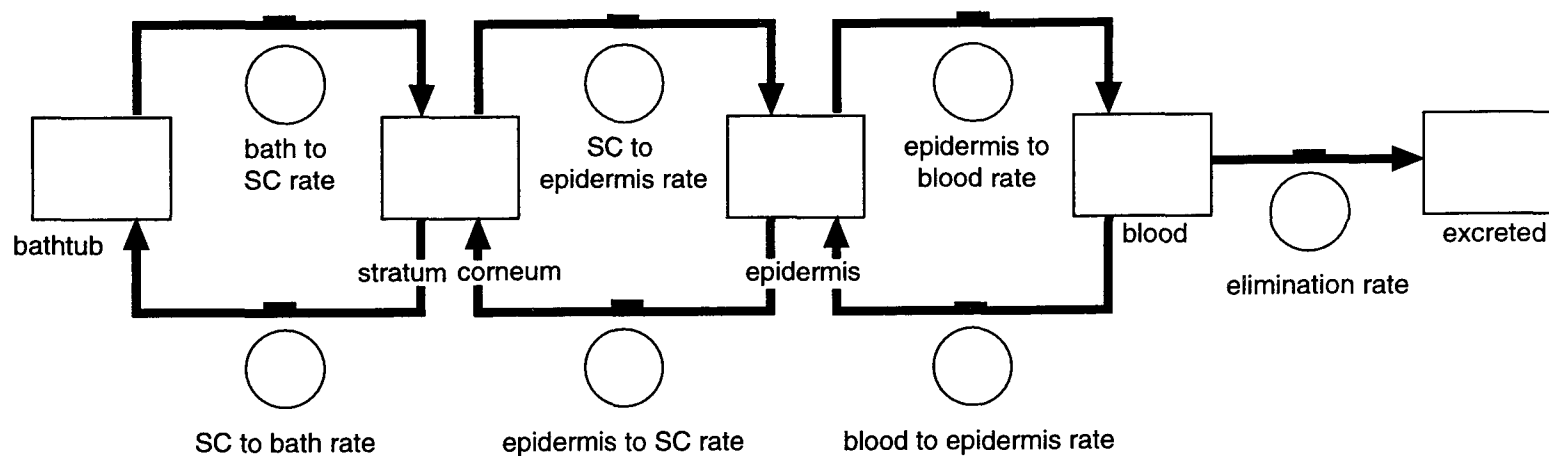
Source: Shatkin and Brown 1991

Table 2-4. Parameters Used in the Shatkin and Brown PBPK Model of Dermal Absorption of Ethylbenzene

Parameter[a]	Value	Reference
Stratum corneum/water partition coefficient (K_m)	NG	Calculated from Roberts et al. 1975
Stratum corneum diffusion coefficient (D_{sc})	NG	Calculated from Guy and Maibach 1984
Skin surface (adult hands and forearms) (adult body)	320 cm^2 NG	Dutkiewicz and Tyras 1967, 1968 Guy and Maibach 1984
Skin surface (infant)	1,900 cm^2	Guy and Maibach 1984
Epidermis diffusion coefficient (D_e)	3.6×10^{-4} cm^2/min	Scheuplein 1969, 1976
Stratum corneum thickness (H_{sc})	0.004 cm	Blank and Scheuplein 1969
Epidermis thickness (H_e)	0.02–0.1 cm	Blank and McAuliffe 1985; Blank and Scheuplein 1969; Guy et al. 1982
Epidermal blood flow (F_{eb}) (adult at rest)	280 mL/min–m^2	Wade et al. 1962
Epidermal blood flow (F_{eb}) (adult, heavy exercise)	4,000 mL/min–m^2	Rowell 1986
Epidermis/blood partition coefficient (K_{eb})	2.75	Shatkin and Brown 1991
Stratum corneum/epidermis partition coefficient ($K_{sc/e}$)	NG	Shatkin and Brown 1991
Blood volume (V_b) (adults)	5,000 mL	Shatkin and Brown 1991
Blood volume (V_b) (infants)	693 mL	Shatkin and Brown 1991
Fat in blood	0.7–0.9%	Brown and Hattis 1989
Fat in stratum corneum	3–6%	Raykar et al. 1988
Fat in epidermis	2–2.5%	Scheuplein 1976
Elimination rate constant (K_e)	0.1 min^{-1}	Hagemann 1979
Octanol/water partition coefficient (K_{ow})	2,230	Published value (reference not cited by authors)

[a]Taken from Shatkin and Brown 1991. All parameters used were either taken from published experimental work of others or calculated from previously reported mathematical relationships.

NG = value not given

In order to test the model, simulations were run using the conditions as in the experiments of Dutkiewicz and Tyras (1967, 1968) (i.e., 1-hour immersion of adult male hands in an aqueous solution). The model indicated that the skin compartments reach steady-state more rapidly than the blood compartment. Storage capacity was shown to be stratum corneum < epidermis < blood. Varying the model parameters in Table 2-4 revealed that the total amount of the chemical entering the body was sensitive to changes in epidermal blood flow. Increasing epidermal thickness and stratum corneum fat decreased the total absorbed, but increasing blood fat had no effect. Overall, the model predicts that thicker, fattier skin will provide some protection from dermal absorption of chemicals, while an increase in epidermal blood flow would increase absorption.

Validation of the model. In order to validate the model, the predicted results were compared to the experimental results of Dutkiewicz and Tyras (1967, 1968) in which male volunteers immersed their hands into a solution of 151 mg/L ethylbenzene for 1 hour. The model predicted 94% of the actual absorbed ethylbenzene dose in the human volunteers. Statistical analysis (e.g., 95% confidence limits or standard deviations of the mean) of the experimental results for the participants in the Dutkiewicz and Tyras (1967, 1968) studies were not reported by the authors and may have some impact on the accuracy of the model predictions.

Target tissues. No specific target tissues were considered in this model.

Species extrapolation. Since human data were used for validation, no species extrapolation was conducted.

Interroute extrapolation. The authors compared the inhalation, oral, and dermal dose of ethylbenzene in adults and infants that could be predicted from exposure to contaminated water in the household environment. For ethylbenzene, the dermal dose in the adult is somewhat greater than the oral or inhalation dose, based on scenarios of bathing, washing dishes, etc. In addition, the model predicts that the dermal dose (mg/kg body weight) absorbed by an infant through bathing would be greater than the adult dermal dose after whole body exposure to the same contaminated water. The authors note that since the wholebody exposures were modeled using parameters for absorption of ethylbenzene through the skin of the forearm, the predictions may be overestimations, and may require some correction.

2.4 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

Aromatic hydrocarbons such as ethylbenzene may only be available for intracellular metabolism and interaction if they are dissolved in aqueous solution (Sikkema et al. 1995). The movement of the chemical into the cell is thought to proceed passively from the aqueous phase, through partitioning of the compound into the lipid bilayer of the cell membrane. As a result, changes in the structure and integrity of the cell membrane may occur (see Section 2.4.2, Mechanisms of Toxicity). This, in turn, may affect membranebound enzyme activity. Evidence for this comes from the work of Engelke et al. (1993), which showed that accumulation of ethylbenzene in the microsomal membranes from pig liver altered the reduction kinetics of cytochromes P-450 and b_5. There is no evidence that the pharmacokinetic mechanisms differ in children compared to adults.

2.4.2 Mechanisms of Toxicity

Ethylbenzene has been shown to exert adverse central nervous system effects on both humans (Yant et al. 1930) and animals (Biodynamics 1986; Cragg et al. 1989; Molnar et al. 1986; Tegeris and Balster 1994; Yant et al. 1930). Although there is no specific data, there is nothing to suggest that the mechanism of toxicity is different in children compared to adults. *In vivo* animal studies of ethylbenzene toxicity at the cellular level indicate that changes in brain levels of dopamine and other biochemical alterations, and in evoked electrical activity in the brain may be involved in ethylbenzene central nervous system toxicity (Andersson et al. 1981; Frantik et al. 1994; Mutti et al. 1988; Romanelli et al. 1986).

In vitro studies of the mechanism of toxicity have focused on the effect of ethylbenzene on cell membranes, particularly that of the astrocyte (Engelke et al. 1993; Naskali et al. 1993, 1994; Sikkema et al. 1995; Vaalavirta and Tahti 1995a, 1995b). In a review by Sikkema et al. (1995), changes in the structure and integrity of the cell membrane after partitioning of ethylbenzene into the lipid bilayer may be a mechanism of toxicity. Changes in the integrity of the cell membrane may subsequently affect the function of membrane, particularly as a barrier and in energy transduction, and in the formation of a matrix for proteins and enzymes. Engelke et al. (1993) showed that incubation of pig liver microsomes with ethylbenzene caused an accumulation of ethylbenzene in the rnicrosomal membrane, which in turn increased the fluidity of the membrane. Although incubation of the microsomal membranes with ethylbenzene did not

change the content of cytochrome P-450 or cytochrome b, content, or the activities of NADPH-cytochrome P-450 reductase or NADH-cytochrome b_5 reductase, a change in the reduction kinetics of these enzymes was observed. The authors proposed that the observed change in kinetics may be due to a rearrangement of the cytochrome P-450 molecules in the microsomal membrane as a result of the accumulation of ethylbenzene in the membrane.

The work of Vaalavirta and Tahti (1995a, 1995b) and Naskali et al. (1993, 1994) has investigated the effect of ethylbenzene on the membrane of the rat astrocyte, as an *in vitro* model for the membranemediated effects of solvents on the central nervous system. Cultured astrocytes from the cerebella of neonatal Sprague-Dawley rats were sensitive to the effects of ethylbenzene, as measured by the inhibition of activity of Na+, K+-ATPase, and Mg++-ATPase (Vaalavirta and Tahti 1995a, 1995b). This effect was found to be dose-dependent (Naskali et al. 1994). Inhibition of these membrane-bound enzymes that regulate the ion channels of the membrane may disturb the ability of the cells to maintain homeostasis. Experiments with rat synaptosome preparations, similar to those using microsomal preparations by EngeIke et al. (1993), showed that membrane fluidity was increased after exposure to ethylbenzene. ATPase and acetylcholinesterase activity were also decreased, as seen in the astrocyte preparations.

2.4.3 Animal-to-Human Extrapolations

Species differences have been shown for ethylbenzene metabolism. In humans exposed via inhalation, the major metabolites of ethylbenzene are mandelic acid (approximately 70%) and phenylglyoxylic acid, which are excreted in the urine (approximately 25%) (Bardodej and Bardodejova 1970; Engstrom et al. 1984). Evidence indicates that the initial step in this metabolic pathway is oxidation of the side chain of ethylbenzene to produce 1-phenylethanol. In rats exposed by inhalation or orally to ethylbenzene, the major metabolites were identified as hippuric and benzoic acids (approximately 38%), 1-phenylethanol (approximately 25%), and mandelic acid (approximately 15-23%), with phenylglyoxylic acid making up only 10% of the metabolites (Climie et al. 1983; Engstrom 1984; Engstrom et al. 1985). In rabbits, the most important metabolite is hippuric acid, which is probably formed by oxidative decarboxylation of phenylglyoxylic acid (El Masri et al. 1958). Rabbits have been shown to excrete higher levels of glucuonidated metabolites than do humans or rats (El Masri et al. 1956; Smith et al. 1954a, 1954b). Thus, there are no animal models of ethylbenzene metabolism that are completely consistent with human metabolism. However, of the experimental models investigated, rats appear to be a more appropriate model than rabbits.

Models of the pharmacokinetic mechanisms and mechanisms of toxicity of ethylbenzene have focused on cellular processes (see Sections 2.4.1 and 2.4.2, above). In these, humans and animals appear to be similar. Although some species differences exist with respect to toxicity, adverse effects observed after ethylbenzene exposure in both humans and animals seem to be similar in scope (i.e., respiratory, hepatic, renal, and neurological). Rats may be more sensitive than mice or rabbits (Cragg et al. 1989; NTP 1992). Thus, the rat may be the most appropriate animal model for studying the mechanism of toxicity of ethylbenzene as it relates to human health effects assessment.

2.5 RELEVANCE TO PUBLIC HEALTH

Overview.

Evidence from the reviewed literature has shown that ethylbenzene produces many adverse effects to both humans and laboratory animals. Clinical observations in humans and observations in animals indicate that the primary symptoms resulting from acute-duration exposure to ethylbenzene are manifested as neurological and respiratory depression and eye and throat irritation. Several studies suggest that target organs of ethylbenzene toxicity, identified in animals but not in humans, may be the liver, kidney, and hematopoietic system. These results, however, are inconclusive (particularly regarding dose-response data) given the weaknesses present in many of these studies.

Ethylbenzene is widely distributed in the environment. The exposure route of most concern to the general public is low-level inhalation exposure over long periods of time. This is due to the direct release of ethyl-benzene into the air by the burning of fossil fuels or industrial processes, and partitioning into the air from other media (e.g., soil, surface water). This partitioning of ethylbenzene into the air or water would play a role in exposure to populations living near hazardous waste sites. However, in all likelihood, the most significant exposure from ethylbenzene at an uncontrolled hazardous waste site would be due to soil contact due to ethylbenzene's affinity for soil organic matter. In addition to inhalation exposure, ingestion of ethyl-benzene may also be a cause of concern because trace amounts have been found in many open water supplies. This concern would be greater for those populations living near hazardous waste sites or gasoline spill sites in which water supplies have been contaminated. Issues relevant to children are explicitly discussed in Sections 2.6, Children's Susceptibility, and 5.6, Exposures of Children.

Minimal Risk Levels for Ethylbenzene.

Inhalation MRLs.

No acute-duration inhalation MRL has been derived for ethylbenzene due to a lack of appropriate data. With regard to human data, Cometto-Muniz and Cain (1995) measured eye irritation and odor thresholds for ethylbenzene. Testing sessions were for 1-2 hours starting with the highest concentration of the chemical being tested. The concentration of the compound in the headspace of each bottle was measured by gas chromatography. Eye irritation thresholds were well above odor thresholds, and eye irritation was observed at 10,000 ppm, whereas odor threshold was at 9 ppm. An eye irritation threshold/odor threshold ratio of 1,333 ppm was reported for ethylbenzene. However, this study did not describe effects that would have an adverse impact on human health, thus making it inappropriate for use in deriving an MRL.

Yant et al. (1930) observed up to six volunteers who were exposed by inhalation to 0.1, 0.2, or 0.5% (1,000, 2,000, or 5,000 ppm) ethylbenzene. At the lowest level, the volunteers reported eye irritation and burning and profuse lacrimation which gradually decreased with continued exposure. Upon entering a chamber with an ethylbenzene concentration of 2,000 ppm, the volunteers experienced severe eye irritation, throat irritation, and chest constriction. One volunteer exposed for 5 minutes to 2,000 ppm experienced vertigo. Four volunteers exposed to 2,000 ppm for 6 minutes experienced dizziness upon leaving the chamber. Three volunteers exposed to 5,000 ppm reported extreme irritation of the eyes, nose, and throat. Although this study described adverse effects of acute-duration inhalation exposure to ethylbenzene in humans, the limitations of the study include use of a few subjects per dose group, lack of data on chemical purity, and lack of control data and other study details, making the study inappropriate for use in deriving an MRL.

Other studies of acute-duration human inhalation exposure to ethylbenzene involved mixtures of solvents (Koren and Devlin 1992), evaluated genotoxic responses (Holz et al. 1995), or used ethylbenzene as a challenge agent to determine respiratory response after exposure to another chemical (Moscato et al. 1987).

•An MRL of 1 .0 ppm has been derived for intermediate-duration inhalation exposure (15-364 days) to ethylbenzene.

The intermediate-duration inhalation MRL of 1 .0 ppm was derived from a NOAEL value of 97 ppm for developmental effects in Wistar rats following inhalation exposure (Andrew et al. 1981). The ratio of the

blood/gas partition coefficients was assumed to be 1. The resulting adjusted NOAEL, 97 ppm, is equal to the human equivalent concentration (HEC) because the ratio of the blood/gas partition coefficients was assumed to be 1. The resulting $NOAEL_{(HEC)}$ 97 ppm, was then divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans after adjusting for the human equivalent concentration, and 10 for human variability) to yield the MRL value of 1 .0 ppm (see Appendix A). No adjustment for intermittent exposure was made because the pharmacokinetics of ethylbenzene indicate that the effects are most likely concentration-dependent and not duration-dependent. The choice of the NOAEL of approximately 100 ppm observed in Andrew et al. (1981) is supported by other studies. In Fischer 344/N rats, administration of 99.4 ppm ethylbenzene for 13 weeks produced no effect on absolute and relative lung or liver weight; administration of 246 ppm for the same duration caused significant increases in these parameters (NTP 1992). In a companion study, rabbits exposed to ethylbenzene using the same paradigm showed no effect on relative liver weight at 99 ppm, but increased absolute and relative liver weight at 962 ppm (Andrew et al. 1981). Although deficient in experimental details, studies reported by Ungvary and Tatrai (1985) support a NOAEL of approximately 100 ppm. In rats, exposure during gestation to ethylbenzene for 24 hours/day for 9 days at doses ranging from 138 to 552 ppm resulted in fetal resorption and retardation of skeletal development in surviving fetuses (Ungvary and Tatrai 1985). Increased incidence of extra ribs and anomalies of the urinary tract were observed at the 552 ppm dose level. No effects were observed after exposure to 138 ppm for 6 hours/day for 9 days (Ungvary and Tatrai 1985). Mice exposed to 115 ppm ethylbenzene during gestation demonstrated an increased incidence of anomalies of the urinary tract (Ungvary and Tatrai 1985). The nature of the renal malformation was not characterized and no maternal toxicity was reported. In addition, reduction in the weight of female fetuses was reported in rabbits exposed to 115 ppm during gestation (Ungvary and Tatrai 1985). Cragg et al. (1989) observed sporadic salivation in Fischer 344 rats exposed to 382 ppm ethylbenzene for 4 weeks.

No chronic-duration inhalation MRL was derived for ethylbenzene due to a lack of appropriate studies. Bardodej and Cirek (1988) monitored 200 male ethylbenzene production workers for 20 years to determine possible hazards to human health. Hematologic tests showed no detectable deviation from normal physiological limits in the parameters tested. No liver lesions or significant difference in liver function tests were reported between exposed and nonexposed humans. The authors reported that ethylbenzene concentrations in "open-type" production plants fall far below the Czechoslovakian MAC limits of 200 mg/m^3 and 1,000 mg/m^3 for average whole-shift and peak-shift concentrations but failed to report more specific concentrations. According to the authors, no incidences of cancer were reported for the last 10 years in this industrial facility in which workers were exposed to ethylbenzene. Due to the lack of quantitative exposure data, this study was not appropriate for use in the derivation of an MRL.

Angerer and Wulf (1985) and Etkina and Etkina (1995) evaluated long-term health effects of inhalation exposure of humans to mixtures of chemicals, including ethylbenzene. The confounding effects of exposure to more than one chemical make these studies inappropriate for use in deriving a chronic-duration inhalation MRL.

The NTP-sponsored 2-year toxicology and carcinogenesis studies of ethylbenzene in Fischer 344/N rats and B6C3F$_1$ mice provide the only animal data for ethylbenzene after chronic-duration exposure. Male and female rats and mice exposed to 0, 75, 250, or 750 ppm ethylbenzene by inhalation for 5 days a week, 6 hours a day for 104 weeks showed adverse health effects. Male rats exposed to 250 and 750 ppm showed body weights lower (<10%) than controls from week 20. Mean body weights of female rats were generally lower (<10%) than controls groups from week 20 and during the second year of the study. Pathological findings in male rats exposed to 750 ppm ethylbenzene showed incidences of renal tubule adenoma and adenoma or carcinoma (combined) significantly greater than incidences in the control group. An extended evaluation of the kidneys showed significant increases in incidences of renal tubule adenoma and renal tubule hyperplasia in both male and female rats exposed to 759 ppm ethylbenzene. In males exposed to 750 ppm, the incidence of renal tubule adenoma or carcinoma (combined) was significantly increased. The severity of nephropathy was increased in both male and female rats exposed to 750 ppm ethylbenzene. The incidence of interstitial cell adenoma in males exposed to 750 ppm was significantly greater than in control group and slightly exceeded the historical control range for inhalation studies. The incidence of bilateral testicular adenoma was also significantly increased in males exposed to 750 ppm. Adenoma in the testes was observed in 36 of 50, 33 of 50,40 of 50, and 44 of 50 male rats exposed to 0, 75, 250, and 750 ppm, respectively. Mean body weights of female mice exposed to 75 ppm were greater (< 10%) than those of controls from week 72 until the end of the study. No clinical findings attributed to ethylbenzene exposure was reported. The incidences of alveolar/bronchiolar adenoma and alveolar/ bronchiolar adenoma or carcinoma (combined) were significantly greater in males exposed to 750 ppm than in the controls but were within the NTP historical control range. The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly greater in female mice exposed to 750 ppm than in the control group but were within the historical control ranges. In male mice, there was a spectrum of non-neoplastic changes in the liver related to ethylbenzene exposure, including syncytial alteration of hepatocytes, hepatocellular hypertrophy, and hepatocyte necrosis. In 750 ppm female mice, the incidence of eosinophilic focus was significantly increased compared to that of the control group. In female mice exposed to 250 and 750 ppm ethylbenzene, the incidences of hyperplasia of the pituitary gland pars distalis were significantly greater than those in the control group. The incidences of thyroid gland follicular cell hyperplasia were significantly greater in both males and females exposed to 750 ppm compared to controls. Although this study is well defined, it is not appropriate for use in deriving an MRL

because the NOAEL for significant non-neoplastic effects (hepatic and renal) is 250 ppm, which is greater than the NOAEL for intermediate-duration exposure.

Oral MRLs.

No acute-, intermediate-, or chronic-duration oral MRLs were derived for ethylbenzene due to a lack of appropriate data.

No studies describing acute-duration oral exposure of humans to ethylbenzene were found in the literature. Ungvary (1986) evaluated the effect of oral exposure to ethylbenzene on the estrous cycle of rats. In the fifth cycle following four normal cycles, oral doses of 500 and 1,000 mg/kg ethylbenzene were given to CFY rats in the morning of estrus, two dies&uses and proestrus. At 3:00, 4:30, and 6:00 p.m. on the day of expected proestrus, the animals were bled. Estradiol, progesterone, and luteinizing hormone levels were determined. Vaginal smears were checked daily. The uterus, ovary, and liver were examined histologically. Oral administration of 500 and 1,000 mg/kg ethylbenzene to female rats in the morning of estrus, two diestruses and proestrus decreased the peripheral hormone levels and blocked the estrous cycle during diestrus. The study limitations include lack of study details, including number of animals, and statistical analysis of data. Another study (Fouchecourt and Riviere 1996) looked at the effect of acuteduration oral exposure of animals to mixtures containing ethylbenzene.

No studies describing intermediate-duration oral exposure of humans to ethylbenzene were found in the literature. Wolf et al. (1956) evaluated the toxicity of certain alkylated benzenes and benzene, and the hazards associated with their use. Groups of 10 female rats were exposed via stomach tube to 0, 13.6, 136,408, or 680 mg/kg ethylbenzene in olive oil 5 times a week for 6 months. A group of 20 rats served as controls and were fed doses of 2.3 mL olive oil emulsified in acacia solution and kept on the same schedule as the treated rats. Hematological examinations determined on selected animals of each group after 20, 40, 80, and 130 doses included total erythrocytes and leukocytes, hemoglobin content as well as a differential blood cell count. Histopathological changes included cloudy swelling of parenchymal cells of the liver and of the kidney tubular epithelium. There were slight effects in the liver and kidney weights (no detail provided). No data were shown on the hematological examination of animals. Study limitations include use of only one sex, and failure to report purity of ethylbenzene. In addition, the study parameters were not well defined. No statistical analyses of the results were performed. Other studies (Fouchecourt and Riviere 1996; Hong et al. 1991) looked at the effect of intermediate-duration oral exposure of animals to mixtures containing ethylbenzene.

No studies describing the health effects of chronic-duration oral exposure of humans to ethylbenzene were found in the literature. The one chronic-duration oral study in animals that was found in the literature described cancer data in Sprague-Dawley rats (Maltoni et al. 1985). MRLs are not derived from cancer data.

Death. No deaths have been reported in humans following ethylbenzene exposure alone, but death has occurred in laboratory animals following acute- or chronic-duration exposure to high levels of ethylbenzene administered via the inhalation, oral, and dermal routes (Biodynamics 1986; Cragg et al. 1989; Ivanov 1962; NTP 1996; Smyth et al. 1962; Wolf et al. 1956). The concentrations of ethylbenzene necessary to cause death in animals have been shown to be relatively high (1,200-13,367 ppm, inhalation exposure; 3,500-4,728 mg/kg/day, oral exposure; 15,433 mg/kg/body weight, dermal exposure). Given this information, death in humans resulting from chronic, low-level exposure to ethylbenzene is unlikely.

Systemic Effects.

Respiratory Effects. Moderate upper respiratory irritation accompanied by chest constriction has been reported in humans exposed by inhalation to high doses (2,000 ppm) of ethylbenzene (Yant et al. 1930), but not to lower doses (Moscato et al. 1987). Animal studies support these findings and show more severe effects with increased doses (De Ceaurriz et al. 1981; Nielsen and Alarie 1982; Yant et al. 1930). The available data on adverse respiratory effects associated with ethylbenzene exposure in animals, coupled with the limited data available on humans, suggest that severe respiratory effects in humans could result following inhalation exposure to high doses of ethylbenzene. Respiratory effects from low-level exposure, such as that found in the air, would be less likely.

Cardiovascular Effects. No cardiac effects have been reported in humans exposed to ethylbenzene alone. In Russian preschool children, exposure to mixed air pollution (including ethylbenzene) was associated with increased incidence of defects of the cardiovascular system (Etkina and Etkina 1995). Studies in animals show no adverse histopathological effects after intermediate-duration inhalation exposure to concentrations up to 2,200 ppm (Cragg et al. 1989; NTP 1992; Wolf et al. 1956) or after chronic-duration inhalation exposure to concentrations up to 750 ppm (NTP 1996). Based on the data available, adverse cardiovascular effects from low-level chronic-duration exposure to ethylbenzene in the air, drinking water, or soil would be unlikely.

Gustrointestinul Effects. No studies were found describing gastrointestinal effects in humans after exposure to ethylbenzene alone. Etkina and Etkina (1995) examined the health of preschool children exposed to chemical mixtures in Russia. The main air pollutants included dust, carbon monoxide, nitric oxides, sulfur oxides, hydrogen sulfide, ammonia, hydrogen chlorous (probably HC1), sulfates, formaldehyde, benzene, toluene, xylene, ethylbenzene, phenol, benzyn (probably volatile hydrocarbons), and hydrocarbon chloride (probably chlorinated hydrocarbons). Exposure to air pollution was associated with increased incidence of defects of the digestive systems. In animals, no adverse histopathological effects were noted in the gastrointestinal system after inhalation exposure to concentrations of ethylbenzene up to 1,610 ppm (Cragg et al. 1989; NTP 1992). However, chronic-duration inhalation exposure of rats and mice to 750 ppm had no adverse effect (NTP 1996).

Hematological Effects. Studies using several species of laboratory animals exposed to ethylbenzene indicate that of the species tested, only rats are susceptible to ethylbenzene-induced hematological effects following inhalation exposure (i.e., increased platelet counts and total leukocyte counts) (Cragg et al. 1989). Other studies indicate no adverse hematological effects after inhalation exposure at equivalent or higher doses (NTP 1992; Wolf et al. 1956). Because of these observed interspecies differences following inhalation exposure and a lack of data following oral and dermal exposures, it is unknown whether hematological effects might occur in humans following exposure to ethylbenzene.

Musculoskeletul Effects. No musculoskeletal effects in humans have been reported in the available literature. Inhalation studies in animals have indicated that exposure to ethylbenzene results in impaired motor coordination, but this may be due more to central nervous system effects that musculoskeletal effects (Tegeris and Balster 1994). Histopathological examination of bone tissue in animals after intermediateduration inhalation exposure to concentrations up to 1,610 ppm showed no adverse effects (Cragg et al. 1989; NTP 1992). Therefore, based on the limited data available, it seems unlikely that musculoskeletal effects would be of concern after low-level chronic-duration exposure to ethylbenzene.

Hepatic Effects. No hepatotoxic effects in humans have been reported in the available literature. Inhalation studies in animals suggest that biochemical changes, changes in liver weight, and histopathological alterations in the liver may be related to dose and duration of exposure to ethylbenzene (Biodynamics 1986; Cragg et al. 1989; Elovaara et al. 1985; NTP 1992, 1996; Toftgard and Nilsen 1982 Wolf et al. 1956). These biochemical changes are accompanied by hepatic hypertrophy, with increased microsomal enzyme activity (Elovaara et al. 1985; Fouchecourt and Riviere 1996). These results are

supported by an intraperitoneal study in rats that demonstrated marked increases in liver enzyme activity (Pyykko et al. 1987). Similar hepatic alterations in mice and rats exposed orally and by inhalation suggest that these effects might occur in humans, but no definitive conclusions can be drawn given the weaknesses of some of the studies, as outlined earlier (Biodynamics 1986; Cragg et al. 1989; Elovaara et al. 1985; Toftgard and Nilsen 1982; Wolf et al. 1956). Despite these weaknesses, the data suggest that humans exposed to ethylbenzene in high concentrations, particularly individuals with compromised liver function, may be at increased risk for ethylbenzene-induced hepatic effects.

Renal Effects. Renal effects, manifested as enzyme changes, increases in organ weight, and tubular swelling or hyperplasia, have been observed in rats and mice (Andrew et al. 1981; Biodynamics 1986; Cragg et al. 1989; NTP 1992, 1996; Toftgard and Nilsen 1982; Wolf et al. 1956). These studies suggest that renal effects may occur in humans exposed to high doses of ethylbenzene. However, significant weaknesses exist in several of these studies. Despite these weaknesses, the data suggest that humans exposed to ethylbenzene in high concentrations, particularly individuals with compromised kidney function, may be at increased risk for ethylbenzene-induced renal effects.

Endocrine Effects. In Russian preschool children, exposure to mixed air pollution (including ethylbenzene) was associated with endocrine effects (Etkina and Etkina 1995). Hormonal imbalance was observed in often ill children which was manifested in increased concentrations of thyrotropin (thyroid-stimulating hormone or TSH), adrenocorticotrophic hormone (ACTH), growth hormone (GH), cortical, and T4. Frequent illnesses did not influence plasma concentration of T3. Children exposed to moderate pollution were observed to have increased activity of endocrine agents, having pronounced diabetogenic effect and catabolic direction of metabolic processes. Children exposed to intense air pollution showed decreased GH, ACTH, T4, cortisol, TSH, and T3. The analysis of interhormonal coefficients showed that exotoxicants disturb interrelations between the central and peripheral endocrine organs, distort interhypophyseal and intrathyroid connections. Studies in animals indicate no adverse histopathological changes to the tissues of endocrine organs after acute- or intermediate-duration inhalation exposure to concentrations up to 2,200 ppm (Cragg et al. 1989; NTP 1992; Wolf et al. 1956). Tissues examined included adrenal, pancreas, pituitary, thyroid, and parathyroid glands. However, mice (but not rats) exposed to 750 ppm ethylbenzene for 2 years exhibited hyperplastic changes in the thyroid gland (NTP 1996). No evaluations of the effect of ethylbenzene on endocrine function were found in the literature. However, based on the limited data available, it seems unlikely that exposure to ethylbenzene at levels found in the environment would cause adverse effects on the endocrine system of the exposed population.

Ocular Effects. Ethylbenzene vapors have been shown to cause ocular irritation, burning, and lacrimation in exposed individuals (Cometto-Muniz and Cain 1995; Thienes and Haley 1972; Yant et al. 1930). Animal studies show similar effects (Biodynamics 1986; Cragg et al. 1989; Tegeris and Balster 1994; Yant et al. 1930). These effects occurred at relatively high concentrations. Thus, ocular irritation would be of concern to populations exposed to high concentrations of ethylbenzene in the air, such as in the occupational setting, but would not likely be of concern at exposure levels found in the environment.

Body Weight Effects. No effects on body weight have been reported in humans after exposure to ethylbenzene alone. In Russian preschool children, exposure to mixed air pollution (including ethylbenzene) was associated with inhibited physical development marked by lower weights (no data shown) in seldom ill children (Etkina and Etkina 1995). In animals, body weight was not a reliable indicator of ethylbenzene toxicity, even at relatively high exposure concentrations (Andrew et al. 1981; Biodynamics 1986; Cragg et al. 1989; NTP 1992, 1996; Romanelli et al. 1986; Wolf et al. 1956). Thus, it is unlikely that changes in body weight would occur in populations chronically exposed to low levels of ethylbenzene such as those found in the environment.

Metabolic Effects. Metabolic effects reported after exposure to ethylbenzene are limited to induction of liver and kidney enzymes in animals (Elovaara et al. 1985; Toftgard and Nilsen 1982). The data suggest that humans exposed to ethylbenzene in high concentrations, particularly individuals with compromised liver or kidney function, may be at increased risk for ethylbenzene-induced effects on enzyme activity.

Other Systemic Effects. Food consumption has been monitored in animal experiments after ethylbenzene exposure and does not appear to be a sensitive indicator of toxicity (Andrew et al. 1981). No other suitable measures of toxicity were found in the literature.

Immunological and Lymphoreticular Effects. No studies were found that described immunological and lymphoreticular effects of exposure of humans to ethylbenzene alone. In Russian preschool children, exposure to mixed air pollution (including ethylbenzene) was associated with increased allergic diseases (Etkina and Etkina 1995). A dose-dependent inhibition of functional activity of the cellular immunity was mostly manifested in often ill children even at low levels of air pollution. IgM was observed to increase in high levels of pollution. At increased levels of pollution, the children revealed an apparent tendency towards reduction of phagocytic index and an increase in the number of phagocytic neutrophils. Data describing effects of ethylbenzene exposure are limited to animal studies (Andrew et al.

1981; Cragg et al. 1989; NTP 1992, 1996; Wolf et al. 1956). These studies looked at gross appearance; organ weight; and histopathology of the spleen, lymph nodes, bone marrow, and thymus, and reported no adverse effects. No evaluations of immunological function were conducted. However, it seems unlikely that immunological effects would be of concern to populations chronically exposed to low levels of ethylbenzene found in the environment.

Neurological Effects. The principal effect in humans acutely exposed via inhalation to high concentrations of ethylbenzene has been central nervous system toxicity (dizziness, vertigo) (Yant et al. 1930). Complete recovery has been shown to occur following acute exposure. Central nervous system effects have also been observed in animal studies (Biodynamics 1986; Cragg et al. 1989; Molnar et al. 1986; Tegeris and Balster 1994; Yant et al. 1930). Changes in evoked electrical activity in the brain have been observed (Frantik et al. 1994). Biochemical alterations were observed in animal studies in which dopamine depletion in the brain following exposure to high concentrations of ethylbenzene was reported. It was suggested that changes in the dopamine levels and turnover might disturb catecholamine neurotransmission in the brain leading to altered brain function (Andersson et al. 1981; Mutti et al. 1988). However, data are available that show increased dopamine turnover, but brain tissue levels remaining constant in all but one of the regions of the brain examined (Andersson et al. 1981). Recent *in vitro* studies indicate that ethylbenzene exposure alters the metabolic activity of astrocytes and synaptosomes (Naskali et al. 1993, 1994; Vaalavirta and Tahti 1995a, 1995b). Given the available human and supporting animal data, there is considerable likelihood that human populations acutely exposed to high concentrations of ethylbenzene are at risk for developing neurological effects. The neurological effects of long-term exposure of humans to ethylbenzene are unknown.

Reproductive Effects. No studies on the reproductive effects in humans following exposure to ethylbenzene were found. Oral administration of ethylbenzene resulted in blockage of the estrus cycle in female rats (Ungvary 1986). This study had many weaknesses (e.g., small number of test animals, no statistical analysis) that prevent definitive conclusions from being drawn. Decreased fertility in female rats was reported following inhalation exposure to ethylbenzene but was not considered to be significant by the authors (Andrew et al. 1981). In addition, increased postimplantation death was reported in rats, and abortions were observed in rabbits, but no effects were seen in mice (Ungvary and Tatrai 1985). The data are insufficient to eliminate the possibility of female reproductive effects. Data describing histopathological evaluation of male reproductive tissues are primarily negative (Biodynamics 1986; Cragg et al. 1989; NTP 1992). Wolf et al. (1956) reported degeneration of the germinal epithelium in one monkey and

one rabbit, but the study had many weaknesses (no numerical data or statistical analysis, insufficient experimental details). Thus, based on the limited data in animals, no conclusion can be drawn concerning the effect of ethylbenzene on reproductive competence in humans. However, the NTP-sponsored 2-year bioassay revealed a significant increase in interstitial cell adenoma and bilateral testicular adenoma in rats, but not mice at 750 ppm ethylbenzene. Thus, male reproductive tissues may be a target for ethylbenzene toxicity.

Developmental Effects. No reports of developmental toxicity following exposure to ethylbenzene in humans were located. The available information from animal studies indicates that inhalation exposure of pregnant rats to ethylbenzene can produce minimal fetotoxic effects at doses that may or may not induce minimal maternal changes (i.e., increased relative liver, kidney, and spleen weights) (Andrew et al. 1981; Ungvary and Tatrai 1985). Mice exposed to 115 ppm ethylbenzene during gestation demonstrated an increased incidence of anomalies of the urinary tract (Ungvary and Tatrai 1985). The nature of the renal malformation was not characterized, and no maternal toxicity was reported. This report contained very few experimental details. Andrew et al. (1981) however, provides a well-defined study in which developmental changes were observed. These developmental effects consisted of an increase in the incidence of supernumerary ribs, which is a non-specific indicator of variation in the development of the skeletal system of rodents. No developmental effects were seen in rabbits exposed to similar levels of ethylbenzene (Andrew et al. 1981). Because of observed interspecies differences, the relevance of these findings with regard to developmental effects in humans cannot be ascertained.

Genotoxic Effects. Holz et al. (1995) reported no increase in sister chromatid exchanges, DNA adduct formation, micronuclei, or DNA single-strand breaks in the peripheral lymphocytes of workers exposed to low levels of ethylbenzene in a styrene plant. NTP (1996) showed no increase in micronucleated peripheral erythrocytes in mice exposed to 750 ppm ethylbenzene for 13 weeks. Another *in vivo* study investigated the genotoxic effects of ethylbenzene and reported no dose-dependent increase in the frequency of micronucleated polychromatic erythrocytes (Mohtashamipur et al. 1985). This study is limited by inadequate sampling time. In addition, the type of clastogenic effect occurring cannot be defined. These data are shown in Table 2-5.

However, the genotoxic potential of ethylbenzene has been investigated primarily using *in vitro* assays in *Salmonella typhimurium* (Dean et al. 1985; Florin et al. 1980; Nestmann et al. 1980; NTP 1986, 1996), *Escherichia coli* (Dean et al. 1985), *Saccharomyces cerevisiae* (Dean et al. 1985; Nestmann and Lee

Table 2-5. Genotoxicity of Ethylbenzene *In Vivo*

Species (test system)	End point	Result With activation	Result Without activation	Reference
Mammalian cells: Mouse (peripheral erythrocytes)	Micronuclei	NA	–	NTP 1996
Human (occupational exposure/ peripheral lymphocytes)	DNA adducts, micronuclei, sister chromatid exchange, DNA strand breaks	NA	–	Holz et al. 1995

NA = not applicable; – = negative results

1983), Chinese hamster ovary cells (NTP 1986, 1996), mouse lymphoma cells (McGregor et al. 1988; NTP 1996), and human lymphocytes (Norppa and Vainio 1983a). Results of these in vitro genotoxicity studies are shown in Table 2-6. The available data indicate that ethylbenzene is not mutagenic in bacteria or yeast cells in the presence or absence of metabolic activation. Ethylbenzene also failed to induce sister-chromatid exchanges and chromosomal aberrations in Chinese hamster cells. A weak positive response was observed when ethylbenzene was tested for sister chromatid exchanges in human lymphocytes (Norppa and Vainio 1983a).

Mutagenicity of ethylbenzene was studied in *S. typhimurium* strains TA97, TA98, TA100, TA1535, TA1537, and TA1538 (Dean et al. 1985; Florin et al. 1980; Nestmann et al. 1980; NTP 1986, 1996) and *E. coli* strains WP_2 and WP_2uvrA (Dean et al. 1985) both in the presence and absence of an S9 mixture. Ethylbenzene was not mutagenic in *S. typhimurium* or *E. coli.*

In gene conversion assays conducted in *S. cerevisiae* strains JDl (Dean et al. 1985), XVl85-14C, and D7 (Nestmann and Lee 1983) no increases in mutation frequencies were detected following application of ethylbenzene. Details of the dose levels tested were not provided.

The potential of ethylbenzene to induce chromosomal aberrations and sister chromatid exchanges was studied in Chinese hamster ovary cells (NTP 1986, 1996). No mutagenic response was observed at dose levels of 75, 100, or 125 mg/L in either assay. However, ethylbenzene was found to be mutagenic at 80 mg/L without metabolic activation, and lethal to cells at 100 mg/L in the mouse lymphoma assays (McGregor et al. 1988). Concentrations of ethylbenzene used ranged from 10 to 160 mg/L. No doseresponse was reported. Ethylbenzene induced a marginal, although significant ($p<0.0l$), increase in sister chromatid exchanges in human lymphocytes but only at the highest dose tested (1,061.6 mg/L), which was also toxic to the cells (Norppa and Vainio 1983a). The relevance of these findings with regard to genotoxic effects of ethylbenzene in humans is not known.

In summary, genotoxicity studies on ethylbenzene have provided negative results in a variety of *in vitro* assays using numerous prokaryotic organisms, S. *cerevisiae,* and Chinese hamster ovary cells and rat liver epithelial cells, and in an *in vivo* assay using mouse bone marrow cells. It has, however, caused a mutagenic effect in mouse lymphoma cells and has been shown to induce a marginal yet significant increase in sister chromatid exchanges in human lymphocytes. Although the majority of the data suggest that

Table 2-6. Genotoxicity of Ethylbenzene *In Vitro*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium (plate incorporation assay)	Gene mutation	–	–	Dean et al. 1985[a]; Florin et al. 1980[b]; Nestmann et al. 1980[c]
S. tymphimurium (plate incorporation assay; strains TA87, TA98, TA100, TA1537; TA1538)	Gene mutation	–	–	NTP 1986[d]
S. tymphimurium (plate incorporation assay; strains TA97, TA98, TA100, TA1535)	Gene mutation	–	–	NTP 1996[d]
Escherichia coli WP$_2$, WP$_2$uvrA	Gene mutation	–	–	Dean et al. 1985[a]
Eukaryotic ornganisms:				
Saccharomyces cerevisiae JD1 gene conversion assay	Gene mutation	–	–	Dean et al. 1985
S. cerevisiae Dy, XV185-14C	Gene mutation	–	ND	Nestmann and Lee 1983
Mammalian cells:				
Mouse lymphoma cells	Gene mutation	ND	+	McGregor et al. *1988*
Mouse lymphoma cells	Gene mutation	ND	+	NTP 1996
Rat liver (RL4) epithelial type cells/chromosome assay	Chromosome damage	–	–	Dean et al. 1985
Chinese hamster ovary cells	Sister chromatid exchange	–	–	NTP 1986
Chinese hamster ovary cells	Sister chromatid exchange	–	–	NTP 1996
Chinese hamster ovary cells	Chromosome damage	–	–	NTP 1996

[a]Concentrations of ethylbenzene tested: 0, 0.2, 2, 20, 500, 2,000 µg/plate (>99% pure)
[b]Concentrations of ethylbenzene tested: 0, 3, 31, 318, or 3,184 µg/plate (0, 0.03, 0.3, 3, or 30 µmole/plate)
[c]Concentrations of ethylbenzene tested: Up to 0.4 mg/plate, a concentration causing lethality
[d]Concentrations of ethylbenzene tested: 0, 10, 33, 110, 333, 666, or 1,000 µg/plate
[e]Weakly positive

ND = no data; – = negative results; + = positive results

ethylbenzene is not mutagenic in most systems, the two studies that did show positive results suggest that ethylbenzene may cause an increase potential for genotoxicity in humans.

Cancer. No association between increased cancer incidence in humans and exposure to ethylbenzene has been reported in current literature. Both chronic bioassays located in the literature showed a significant increase in tumors in rats, either exposed by inhalation (NTP 1996) or orally exposed (Maltoni et al. 1985). The NTP (1996) study provides clear evidence of carcinogenicity in male Fischer 344/N rats exposed to 750 ppm ethylbenzene for up to 2 years, citing the incidence of renal and testicular lesions. Evidence for female rats and male and female B6C3F$_1$ mice is suggestive, but not conclusive. The results from the Maltoni et al. (1985) study, however, are inconclusive, given the weaknesses of the study (e.g., only one dose was tested and no survival data were provided).

In the 1996 Integrated Risk Information System (IRIS 1996) database, EPA has classified ethylbenzene as a Group D agent (Not Classifiable as to Carcinogenicity). This classification applies to those chemical agents for which there is inadequate evidence of carcinogenicity in humans or animals. No potency factor (q$_1$*) or other quantitative estimate of carcinogenicity has been developed by EPA for ethylbenzene. The International Agency for Research on Cancer (IARC) and the National Toxicology Program (NTP) have yet not classified this chemical for carcinogenicity. However, based on the findings of the recent NTP report (NTP 1996), this is likely to change in the near future.

2.6 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate due to maternal exposure during gestation and lactation. Relevant animal and in vitro models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 5.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both pre-natal and post-natal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns and at various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults and sometimes unique enzymes may exist at particular developmental stages (Komori 1990; Leeder and Keams 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in the newborn who has a low glomerular filtration rate and has not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility while others may decrease susceptibility to the same chemical. For example, the fact that infants breathe more air per kilogram of body weight than adults may be somewhat counterbalanced by their alveoli being less developed, so there is a disproportionately smaller surface area for absorption (NRC 1993).

There are no data describing the effect of exposure to ethylbenzene on children or immature animals. Respiratory and eye irritation, and dizziness are the most prevalent signs of exposure to high levels of

ethylbenzene (Yant et al. 1930), and it is expected that children would also exhibit these effects, as well as other effects observed in adults. Minor birth defects have occurred in newborn rats, but not rabbits, whose mothers were exposed by breathing air contaminated with ethylbenzene (Andrew et al. 1981; Ungvary and Tatrai 1985). These defects consisted of urinary tract anomalies, and supernumerary ribs, a frequently observed indicator of variation in the development of the skeletal system in rodents. Supernumerary ribs were observed in the presence of minimal maternal changes. Section 2.2.1.6, Developmental Effects, contains a more detailed discussion of these results. A significant increase in the incidence of extra ribs occurred at only one dose during gestation in the study by Andrews et al. (1981). The report by Ungvary and Tatrai (1985) lacks pertinent experimental details that would strengthen the validity of their results. In addition, it is not known whether these developmental effects would be observed in people. There are no human developmental data. Ethylbenzene has been detected in human breast milk at unspecified concentrations (Pellizzari et al. 1982), but no pharmacokinetic experiments have been done to confirm that it is actually transferred to breast milk in mammals. It is not known if ethylbenzene crosses the placenta.

Since there is no information about health effects in children, it is unknown whether they differ from adults in their susceptibility to health effects from ethylbenzene. There is no specific information about the metabolism of ethylbenzene in children or immature adults. However, since two of the enzyme families responsible for the conjugation and elimination of ethylbenzene are developmentally regulated, it is possible that the activity of these enzymes would differ in children or immature animals compared to adults. In humans, UDP glucuronosyltransferase activity does not reach adult levels until about 6-l 8 months of age, although the development of this activity is isoform specific. Activity of sulfotransferases (which is also isoform specific) seems to develop earlier. The activity of some sulfotransferase isoforms may even be greater during infancy and early childhood than in adulthood (Leeder and Keams 1997).

2.7 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NRC 1989b). The preferred

biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to ethylbenzene are discussed in Section 2.7.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by ethylbenzene are discussed in Section 2.7.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.9, Populations That Are Unusually Susceptible.

2.7.1 Biomarkers Used to Identify or Quantify Exposure to Ethylbenzene

Information on ethylbenzene concentrations in human tissue or fluids is available. Exposure to ethylbenzene can be determined by the detection of mandelic acid and phenylglyoxylic acid in urine or by direct detection of ethylbenzene in whole human blood. The only available study that associated levels of ethylbenzene in human tissue and fluids with health effects was conducted by Angerer and Wulf (1985). In this study, specimens of whole blood from 35 male workers chronically exposed to organic solvents

containing ethylbenzene were analyzed. The mean ethylbenzene concentrations detected from personal air monitoring was 4 ppm, and the corresponding mean concentration of ethylbenzene in the blood samples was 61.4 μg/L. Significant correlations between the concentrations of ethylbenzene in air and blood and leukocyte counts were reported. However, blood lead levels could have been a confounding factor.

The 1982 National Human Adipose Tissue Survey conducted by EPA measured ethylbenzene in 96% of the 46 composite samples analyzed for volatile organic compounds (Stanley 1986). A wet tissue concentration range of not detected (detection limit=2 ng/g) to 280 ng/g was reported, but an average concentration was not provided.

Numerous studies indicate that environmental exposures to ethylbenzene can result in detectable levels in human tissues (Antoine et al. 1986; Cramer et al. 1988; Pellizzari et al. 1982; Wolff 1976; Wolff et al. 1977) and in expired air (Conkle et al. 1975; Engstrom and Bjurstrom 1978; Wallace et al. 1984). Analysis of blood specimens from a test population of 250 patients (Antoine et al. 1986) and composite samples obtained from blood donations of laboratory personnel with potentially low-level exposure (Cramer et al. 1988) indicated ethylbenzene concentrations in the blood to range from below detection limits to 59 ppb. Similarly, ethylbenzene was detected in 8 of 12 milk samples from lactating women living in various urban areas of the United States with high probability of emissions of pollutants (Pellizzari et al. 1982). Subcutaneous fat samples taken from individuals exposed to an average of l-3 ppm ethylbenzene in the workplace contained ethylbenzene levels as high as 0.7 ppm (Wolff 1976; Wolff et al. 1977).

Studies examining the correlation of ethylbenzene concentrations in ambient air with concentrations measured in expired or alveolar air have also been conducted (ConkIe et al. 1975; Engstrom and Bjurstrom 1978; Wallace et al. 1984). Ethylbenzene concentrations in breath samples were reported to correlate well with ethylbenzene concentrations in indoor samples taken with personal air monitors (Wallace et al. 1984). A correlation was also found between ethylbenzene uptake and ethylbenzene concentrations in alveolar air during, but not after, inhalation exposure in human volunteers (Engstrom and Bjurstrom 1978). Rates of ethylbenzene expiration measured in volunteers with no known previous exposure to ethylbenzene ranged from 0.78 μg/hour to 14 μg/hour, with higher rates detected in smokers than in nonsmokers (Conkle et al. 1975).

2.7.2 Biomarkers Used to Characterize Effects Caused by Ethylbenzene

For more information on biomarkers for renal and hepatic effects of chemicals see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (1990) and for information on biomarkers for neurological effects see OTA (1990).

No specific biornarkers of effect for ethylbenzene were identified. Most of the information on humans is from case reports in which the effects are general and non-specific, such as eye and throat irritation and chest constriction (Yant et al. 1930).

There is one study that indicates that the average number of lymphocytes increased (p=0.005) and hemoglobin levels decreased (p=0.001) following exposure of 35 men to ethylbenzene (Angerer and Wulf 1985), but these data were not substantiated in a long-term study on 200 occupationally exposed male workers (Bardodej and Cirek 1988). Given the nonspecificity of these end points and the presence of blood lead levels that could confound the results (Angerer and Wulf 1985), it would be difficult to correlate changes in these parameters with exposure to ethylbenzene.

2.8 INTERACTIONS WITH OTHER CHEMICALS

Interaction of ethylbenzene with carbon monoxide, phenobarbital, and *m*-xylene have been described in numerous studies. Carbon monoxide has been shown to inhibit the *in vitro* hydroxylation of ethylbenzene when the ratio of carbon monoxide to atmospheric oxygen is 2 to 1 (Maylin et al. 1973). Similarly, simultaneous exposure of rats to ethylbenzene and xylenes has produced inhibitory effects on ethylbenzene metabolism as evidenced by a decreased excretion rate of urinary ethylbenzene metabolites (Angerer and Lehnert 1979; Elovaara et al. 1984; Engstrom et al. 1984). Similar metabolic inhibitory effects were seen in female rats intraperitoneally pretreated with ethanol before inhalation exposure to ethylbenzene as evidenced by significantly increased ethylbenzene blood levels compared with animals pretreated with physiological saline (Romer et al. 1986). The authors suggested that increased central nervous system disturbances (e.g., depression) may be expected following simultaneous exposure to ethylbenzene and ethanol. Conversely, pretreatment with phenobarbital has been shown to increase the rate of ethylbenzene oxidation both in vitro (Maylin et al. 1973; McMahon and Sullivan 1966) and *in vivo* in rats (McMahon and Sullivan 1966).

Though no further studies were located that demonstrate specific interactions of ethylbenzene with other chemicals, a number of substances are known to influence the metabolism of many xenobiotics. For instance, the metabolism of ethylbenzene can be markedly altered by inhibitors (e.g., SKF 525A) and inducers (e.g., phenobarbital, described above) of drug-metabolizing enzymes (Gillette et al. 1974) and by the availability of detoxication agents (e.g., glucuronic acid or sulfates) that bind ethylbenzene metabolites and subsequently are excreted from the body. Mono-oxygenases (MOs) are a class of enzymes involved in the detoxication of xenobiotics, including ethylbenzene. Substances that induce MO enzymes may decrease the toxicity of ethylbenzene by increasing the rate of production of its less toxic metabolites. Conversely, MO enzyme inhibitors would be expected to have the opposite effect. Compounds that affect glucuronic acid availability could also affect the excretion rate of ethylbenzene metabolites.

2.9 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to ethylbenzene than will most persons exposed to the same level of ethylbenzene in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters may result in reduced detoxication or excretion of ethylbenzene, or compromised function of target organs affected by ethylbenzene. Populations who are at greater risk due to their unusually high exposure to ethylbenzene are discussed in Section 5.6, Populations With Potentially High Exposure.

Even though ethylbenzene is not known to bioaccumulate, human and animal studies suggest that several factors can contribute to an increased probability of adverse health effects following ethylbenzene exposure (Mackinson et al. 1978). Exposure of individuals with impaired pulmonary function to ethylbenzene in air has been shown to exacerbate symptoms because of ethylbenzene's irritant properties. Because ethylbenzene is detoxified primarily in the liver and excreted by the kidney, individuals with liver or kidney disease might be more susceptible to ethylbenzene toxicity, as would persons taking medications or other drugs (e.g., alcohol) that are known hepatotoxins. Persons with dermatitis or other skin diseases may be at greater risk, since ethylbenzene is a defatting agent and may aggravate these symptoms. Children's susceptibility is discussed in Section 2.6.

In summary, groups that might be more susceptible to the toxic effects of ethylbenzene are individuals with diseases of the respiratory system, liver, kidney, or skin; young children; fetuses; pregnant women; and individuals taking certain medications such as hepatotoxic medications or drugs.

2.10 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to ethylbenzene. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to ethylbenzene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. No information specifically addressing treatment following exposures to ethylbenzene was found.

The following texts provide specific information about treatment following exposures to styrene, a similar compound:

Carl Zenz et al. (eds.) (1994). Occupational Medicine, 3rd edition. Mosby, St. Louis.

Ellenhorn, MJ and Barceloux, DG (eds.) (1988). Medical Toxicology: Diagnosis and Treatment of Human Poisoning. Elsevier Publishing, New York, NY.

2.10.1 Reducing Peak Absorption Following Exposure

Human exposure to ethylbenzene can occur by inhalation, oral, or dermal contact. General recommendations for reducing absorption of ethylbenzene were not found in the literature. Recommendations for reducing absorption of styrene [C_8H_8], a chemically and structurally similar compound to ethylbenzene [C_8H_{10}], are provided here.

The removal of the patient from the source of contamination is an initial priority along with proper ventilation and cardiac monitoring. Recommendations included the removal of all contaminated clothing and thorough washing of exposed areas with green soap and water. If the patient is alert, syrup of ipecac is recommended following ingestions exceeding 2-3 mL/kg. For patients at risk because of obtundation, intubation should precede lavage (Ellenhorn and Barceloux 1988; Zenz 1994). If a smaller quantity has been ingested, a physician should be called immediately. Syrup of ipecac or other means of inducing vomiting is not recommended for ingestion of smaller quantities since ethylbenzene can directly enter the

lungs if swallowed, or if subsequently vomited, due to its low viscosity (32 SUS at 100°F). Once in the lungs, it can cause chemical pneumonitis, pneumonia, and pulmonary edema (Gossel and Bricker 1994).

2.10.2 Reducing Body Burden

Following absorption into the blood, ethylbenzene is rapidly distributed throughout the body. The initial stage of ethylbenzene metabolism in humans is the formation of 1-phenylethanol via hydroxylation of the of the side chain. Further oxidation leads to the formation of mandelic acid and phenylglyoxylic acid, the major urinary metabolites of ethylbenzene in humans. Detoxication pathways generally involve the formation of glucuronide or sulfate conjugates of 1 -phenylethanol or its subsequent metabolites. Urinary excretion is the primary route of elimination of metabolized ethylbenzene. Studies in humans and animals indicate that urinary excretion occurs in several phases, with half-lives of hours. Hence, ethylbenzene and its metabolites have relatively short half-lives in the body, and while some of these metabolites are clearly toxic, substantial body burdens are not expected.

No methods are currently used for reducing the body burden of ethylbenzene. It is possible that methods could be developed to enhance the detoxication and elimination pathways.

2.10.3 Interfering with the Mechanism of Action for Toxic Effects

Aromatic hydrocarbons, such as ethylbenzene, may only be available for intracellular interaction if they are dissolved in aqueous solution (Sikkema et al. 1995). Changes in the structure and integrity of the cell membrane may occur after the chemical molecule dissolves into the lipid bilayer of the membrane. Changes in the integrity of the cell membrane may subsequently affect the function of membrane, particularly as a barrier and in energy transduction, and in the formation of a matrix for proteins and enzymes. The work of Vaalavirta and Tahti (1995a, 1995b) and Naskali et al. (1993, 1994) suggests that changes in the cell membrane caused by ethylbenzene may disturb the ability of the cell to maintain homeostasis. Experiments with rat synaptosome preparations showed that membrane fluidity was increased after exposure to ethylbenzene, accompanied by changes in the activity of membrane-bound enzymes. It is possible that stabilizing the cell membrane so that the ethylbenzene would be unable to enter the lipid bilayer could provide protection against the subsequent toxic effects of the compound.

Ethylbenzene has been shown to exert adverse central nervous system effects on both humans (Yant et al. 1930) and animals (Biodynamics 1986; Cragg et al. 1989; Molnar et al. 1986; Tegeris and Balster 1994; Yant et al. 1930). In vivo animal studies of ethylbenzene toxicity at the cellular level indicate that changes in brain levels of dopamine and other biochemical alterations, and in evoked electrical activity in the brain may be involved in ethylbenzene central nervous system toxicity (Andersson et al. 1981; Frantik et al. 1994; Mutti et al. 1988; Romanelli et al. 1986). Treatment measures aimed at preventing these changes in neurotransmitter levels and electrical activity may serve to lessen or prevent the central nervous system effect of ethylbenzene exposure.

2.11 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of ethylbenzene is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of ethylbenzene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.11.1 Existing Information on Health Effects of Ethylbenzene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to ethylbenzene are summarized in Figure 2-6. The purpose of this figure is to illustrate the existing information concerning the health effects of ethylbenzene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific

Figure 2-6. Existing Information on Health Effects of Ethylbenzene

Human

Animal

● Existing Studies

information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Figure 2-6 graphically describes the existing health effects information on ethylbenzene by route and duration of exposure. Little information concerning humans exposed via inhalation to ethylbenzene is available. Most of the information concerning health effects in humans is reported in occupational studies, which are difficult to interpret given the limitations of the studies (e.g., simultaneous exposure to other hazardous substances, unquantified exposure concentrations, and exposure probably occurring by a combination of routes). No data were available concerning human health effects following oral or dermal exposures to ethylbenzene. Dermal effects in humans are limited to respiratory and/or ocular irritation after exposure to ethylbenzene vapor.

In animals, the lethality of ethylbenzene is documented for all routes of exposure. Systemic health effects following inhalation exposure to ethylbenzene for acute and intermediate durations as well as immunologic, neurologic, developmental, and reproductive effects are also described. Limited data on the health effects resulting from oral or dermal exposure to ethylbenzene were located. No chronic systemic or genotoxic studies were located for the oral, inhalation, or dermal routes of exposure.

2.11.2 Identification of Data Needs

In general, data on the toxic effects of ethylbenzene in humans and animals are limited. In many areas for which studies have been conducted, the lack of reliable data precludes any definitive conclusions from being drawn and the development of corresponding MRLs.

Acute-Duration Exposure. Inhalation exposure of humans to ethylbenzene results in irritation of the eyes and lungs. In addition, neurological effects such as dizziness have been reported in humans following acute-duration exposure to this chemical. Similarly, respiratory and neurological effects have been observed in animals exposed to ethylbenzene via inhalation. However, only one dose level was used in many of the studies; therefore, information on dose-response relationships was not available. Furthermore, data for acute-duration oral exposure to ethylbenzene are lacking. No acute-duration studies were suitable for deriving inhalation or oral MRLs for ethylbenzene. Well conducted acute-duration studies via inhalation and the oral route, using a number of exposure concentrations and well defined protocols would be useful in establishing this dose-response relationship and elucidating any thresholds that may exist for

acute adverse health effects. The potential for brief human exposure to high concentrations of ethylbenzene exists in accidental exposure in the workplace, hazardous waste sites, and gasoline spill sites.

Intermediate-Duration Exposure. No intermediate-duration studies were located for humans exposed to ethylbenzene by the inhalation, oral, or dermal routes. Repeated inhalation exposures of animals to ethylbenzene have been evaluated. Respiratory, hepatic, renal, and neurological effects have been characterized in animals (Andrew et al. 1981; Cragg et al. 1989; Elovaara et al. 1985; NTP 1992). No intermediate-duration studies were suitable for deriving oral MRLs for ethylbenzene. The NTP 90-day inhalation study (NTP 1992) provides additional information on systemic toxicity following inhalation exposure. Additional histopathology data resulting from repeated oral exposure would be particularly useful, since the potential exists for humans to be exposed to ethylbenzene in the drinking water. These data would help confirm that the liver and kidney are target organs and determine sensitive end points in the liver and kidney, which appear to be the target organs following intermediate-duration exposure to ethyl-benzene. There are data (Fouchecourt and Riviere 1996) suggesting that the lung may be a target, but these data need to be confirmed. Presently, one study exists that may suggest the occurrence of histopathological changes in the liver and kidney following repeated oral exposure to ethylbenzene (Wolf et al. 1956). However, these results are marked by limitations in the methodology, the end points monitored, and the reporting of data; therefore, no definitive conclusions can be drawn. Data on intermediate-duration dermal exposure in humans are lacking, and in animals are limited to effects of exposure of mucous membranes to ethylbenzene vapor. Such information would also be useful in determining human health effects, since the potential exists, both in the occupational setting and at hazardous waste sites, for such exposure to occur.

Chronic-Duration Exposure and Cancer. No adverse health effects were seen in a long-term (20 years) inhalation study of 200 workers occupationally exposed to ethylbenzene (Bardodej and Cirek 1988). No chronic-duration dermal studies in animals were located in the literature. One chronic-duration inhalation study (NTP 1996) suggesting evidence of carcinogenicity in B6C3F$_1$ mice and F344/N rats and one chronic-duration oral study (Maltoni et al. 1985) reporting increases in malignant tumors were found. Studies that evaluate the effects of long-term exposures and provide quantitative exposure data would be useful as the potential exists for human populations to be exposed to ethylbenzene from contamination at hazardous waste sites, particularly from oral and inhalation exposures. The data are currently limited to the two studies listed above.

The carcinogenicity of ethylbenzene was investigated in three studies: an epidemiological study of humans occupationally exposed by inhalation (Bardodej and Cirek 1988), an inhalation exposure 2-year bioassay in rats and mice (NTP 1996), and an oral study using rats (Maltoni et al. 1985). The results of both the human epidemiological study and the oral study in rats were inconclusive, given the marked limitations present in both studies (e.g., possible simultaneous exposure to other chemicals in the human study; only one dose group used and no survival data in the animal study). However, the NTP study in rats and mice showed clear evidence of carcinogenicity in male rats, and some evidence of carcinogenicity in female rats and male and female mice (NTP 1996). No carcinogenicity studies following dermal exposure were located in the literature. Since dermal absorption can be a significant route of exposure to the liquid form of ethylbenzene, further studies of the dermal effects of ethylbenzene would be informative.

Genotoxicity. Available human data (Holz et al. 1995) indicate that ethylbenzene may be genotoxic. A weak induction of sister chromatid exchanges in human lymphocytes following ethylbenzene exposure was seen (Norppa and Vainio 1983a). Data are available regarding the genotoxic potential of ethylbenzene from *in vitro* assays in bacteria, yeast, and mammalian cell cultures (McGregor et al. 1988; NTP 1996). These data are summarized in Table 2-6. Although the results generally indicate that ethylbenzene is not genotoxic, marginal genotoxic effects have been reported in some tests. Independent confirmation or refutation of these studies, as well as further genotoxicity studies, especially in mammalian systems, would help provide clarification of these conflicting results. In particular, chromosome aberrations in occupationally exposed persons would provide useful information.

Reproductive Toxicity. No studies on reproductive effects of ethylbenzene by the inhalation or oral routes in humans and few studies using animals were located. The results from one animal study suggest that adverse reproductive effects may occur in animals following oral exposure to ethylbenzene (Ungvary 1986). No reduced fertility was reported in rats exposed to ethylbenzene by inhalation, but the possibility of this occurring was not ruled out. Additional reproductive studies, particularly for the inhalation and oral routes of exposure and involving multigenerational or continuous breeding studies, would help clarify the potential for ethylbenzene to cause adverse reproductive effects in humans.

Developmental Toxicity. No human data on developmental toxicity of ethylbenzene by any route are available. Minor birth defects have occurred in newborn rats, but not rabbits, whose mothers were exposed by breathing air contaminated with ethylbenzene (Andrew et al. 1981; Ungvary and Tatrai 1985). These defects consisted of urinary tract anomalies, and supernumerary ribs, a frequently observed indicator of

variation in the development of the skeletal system in rodents. Supernumerary ribs were observed in the presence of minimal maternal changes. A developmental MRL for intermediate-duration inhalation exposure was determined based on data from the rat study (Andrew et al. 1981). No studies were located that considered subtle developmental effects observed during postnatal development (e.g., behavioral or learning disability). In addition, no studies of postnatal exposure were found. These studies would be helpful in evaluating potential developmental effects in humans exposed to ethylbenzene via inhalation or ingestion. There are no available data concerning dermal exposure to ethylbenzene during development. Since it is possible that women and children may come in dermal contact with liquid ethylbenzene, these additional studies would be useful in more thoroughly evaluating the potential of ethylbenzene to cause developmental effects.

Immunotoxicity. No data are available regarding the immunotoxicity of ethylbenzene in humans or animals by the inhalation, oral, or dermal routes. There are some data to suggest that hematological effects may be seen, but these data are inconsistent (Cragg et al. 1989; NTP 1992). Inhalation and oral exposure studies would be most useful in confirming the potential of ethylbenzene to affect blood cells, since these routes are the major ways by which persons are exposed to ethylbenzene. Dermal sensitization tests may also provide useful data on the likelihood of an allergic response occurring since the potential for skin contact by humans occurs in the workplace and in soil and water at hazardous waste sites.

Neurotoxicity. Acute-duration inhalation studies in humans and animals exposed to ethylbenzene indicate that ethylbenzene causes neurological effects (Andersson et al. 1981; Molnar et al. 1986; Mutti et al. 1988; Romanelli et al. 1986; Tegeris and Balster 1994). Some data are available on possible mechanisms of action through dopamine depletion. No ethylbenzene-related behavioral changes were reported in one study (Yant et al. 1930), but other neurological parameters were not monitored. Studies have been conducted that investigated biochemical changes in the brains of animals following inhalation exposure, and some studies were located regarding histopathological changes following ethylbenzene exposure (Biodynamics 1986; Cragg et al. 1989). Well conducted acute-, intermediate-, and chronicduration studies including functional observation batteries, motor activity, and neurological evaluation across all exposure routes would be useful for confirming these data.

Epidemiological and Human Dosimetry Studies. The few available epidemiological studies on the health effects of ethylbenzene were primarily limited to occupational studies in which quantitative estimates of exposure were lacking and other limitations (e.g., multiple exposure routes, simultaneous

exposure to other hazardous chemicals) were present. Studies using volunteers exposed to low concentrations of ethylbenzene have provided useful information on effects of acute-duration inhalation exposure on the central nervous system (Yant et al. 1930). No studies were available in which humans were exposed orally or dermally to ethylbenzene. Further epidemiological studies conducted in the vicinity of hazardous waste sites containing ethylbenzene or in occupational settings where ethylbenzene is used would provide useful information on the health effects in humans. Data on lung function and neurological effects from these studies would be particularly valuable as these are likely to be targets of ethylbenzene toxicity.

Biomarkers of Exposure and Effect. Sensitive methods are available for determining ethylbenzene and ethylbenzene metabolites in biological tissues and fluids. However, limited data are available associating levels of ethylbenzene in human tissues and fluids with adverse health effects. Hematopoietic changes were shown to correlate with ethylbenzene concentrations in the blood. Additional animal or epidemiological studies evaluating the association between levels in tissue or fluids and adverse health effects would be useful to devise more sensitive and more specific early biomarkers of effect.

Exposure. Exposure to ethylbenzene can be monitored through levels of ethylbenzene in breath, bIood, or tissue, or levels of its metabolites, mandelic or phenylglyoxylic acid in urine. Both of these metabolites are considered to be specific to ethylbenzene (Ogata and Taguchi 1987). The Biological Exposure Index (BEI) for ethylbenzene is 1.5 g mandelic acid/g creatinine in urine (ACGIH 1996). Additional identification of biomarkers of exposure to ethylbenzene are not necessary at this time.

Effect. There are currently no known specific biomarkers of effect for ethylbenzene. Development of methods to identify biomarkers that would indicate toxic effects, and the extent of those toxic effects after exposure to ethylbenzene, would be helpful in managing health effects that occur after significant exposure to ethylbenzene.

Absorption, Distribution, Metabolism, and Excretion. Quantitative and qualitative evidence indicates that ethylbenzene is rapidly and efficiently absorbed by humans following inhalation and dermal exposures. Animal data support these findings and indicate that absorption rates are high following oral exposures as well.

Only one study (Engstrom and Bjurstrom 1978) is available that outlines the distribution of ethylbenzene in humans following inhalation exposure. This study indicates rapid distribution to adipose tissues

throughout the body. Numerous oral and inhalation studies in animals support these results. Ethylbenzene is accumulated primarily in the intestine, liver, kidney, and fat, which provides some basis for ethyl-benzene-induced effects observed in the liver and kidney. No data on distribution of ethylbenzene following dermal exposure were located. Such information would be useful because absorption of liquid ethylbenzene via this route is rapid in humans and because the potential exists in humans for dermal exposure.

The metabolism of ethylbenzene in humans and animals has been studied. Although some differences in the metabolic pattern according to route of exposure, sex, nutritional status, and species have been documented, pharmacokinetic data show no significant differences in metabolism between oral and inhalation routes in either humans or animals. Further studies that correlate these differences in metabolism with differences in health effects would be useful. Data on metabolism following dermal exposure are sparse, because it is difficult to accurately measure absorption of volatile compounds. Additional data on metabolism following dermal exposure would be useful as these exposures could occur both from contaminated soil or groundwater.

Ethylbenzene has been shown to be rapidly eliminated from the body following inhalation exposure (primarily in the urine) in both humans and animals. These studies (Gromiec and Piotrowski 1984; Yamasaki 1984) are sufficient to characterize the elimination of ethylbenzene following inhalation exposure. A small number of studies in animals exposed orally and humans exposed dermally support these findings. Further studies on elimination of ethylbenzene via these exposure routes would be useful, especially because differences in the excretion patterns have been observed with different routes of exposure.

Comparative Toxicokinetics. Quantitative and qualitative variations in the absorption, distribution, metabolism, and excretion of ethylbenzene were observed depending on exposure routes, sex, nutritional status, and species, as previously outlined. Further studies that focus on these differences and their implications for human health would be useful. Additionally, *in vitro* studies using human tissue and physiologically based pharmacokinetic modeling would contribute significantly to the understanding of the kinetics of ethylbenzene, since they would provide information on half-lives and saturation kinetics associated with the metabolism of ethylbenzene.

Methods for Reducing Toxic Effects. No information was found that specifically addressed the reduction of toxic effects after absorption of ethylbenzene. Development of clinical procedures for

minimizing the effects of ethylbenzene on the respiratory, hepatic, and renal systems, and the central nervous system would be useful in situations where significant exposure had occurred.

Children's Susceptibility. There are no data describing the effects of ethylbenzene exposure in children or developing postnatal animals. Data needs relating to development are discussed in more detail above under developmental effects. In order to evaluate whether ethylbenzene presents a unique hazard to children, additional information on the health effects, pharmacokinetics, metabolism, and mechanism of action in children is needed. It is unknown whether children differ from adults in their susceptibility to health effects from exposure to ethylbenzene. Pharmacokinetic studies investigating whether ethylbenzene or its active metabolites cross the placenta or are transferred into breast milk would be useful. Studies to determine whether there are specific biomarkers of exposure in children would be helpful in monitoring the exposure of children to this chemical. In addition, information describing methods of reducing toxic effects and decreasing beody burden in children might be helpful. Child health data needs relating to exposure are discussed in Section 5.8.1, Data Needs: Exposures of Children.

2.11.3 Ongoing Studies

The toxicological significance of the metabolism of alkylbenzenes is currently being investigated by W.1. Backes (Louisiana State University). The objective of this project is to supply information that will aid in the identification of conditions under which individuals might be susceptible to alkylbenzene (including ethylbenzene) toxicity. No other ongoing studies regarding health effects of ethylbenzene exposure were identified in the available literature (FEDRIP 1996).